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# CYTOGENETIC STUDIES IN SOLANUM, SECT. TUBERARIUM

BY *ROBERT LAMM*
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## I. INTRODUCTION.

IN 1931 the Swedish Seed Association, Svalöf, received a collection of potato samples from Bolivia, including diploid, triploid and tetraploid clones. These samples were sent by order of the Swedish explorer, the late Prof. E. NORDENSKIÖLD. In a paper entitled »Studies on meiosis in diploid and triploid *Solanum tuberosum* L.» MÜNTZING (1933 a) published the results of his investigations at the Cyto-Genetic Department of the Seed Association on this potato material.

During the years 1932—1934 I was working as assistant to Prof. A. MÜNTZING and then had the opportunity of following his investigations. His potato material was also most kindly put at my disposal for further investigations.

The Svalöf collection of South American potato species was further supplemented in 1934 by a great number of samples collected by Dr. C. HAMMARLUND in Bolivia and Peru (HAMMARLUND, 1943). By the chief of the Potato Department of the Seed Association, Dr. O. TEDIN, I was kindly permitted to choose from this collection any samples that might be of interest for my own investigations.

After a visit in 1937 to the Kaiser Wilhelm-Institut für Züchtungsforschung, Müncheberg (Mark), the potato-cytologist of this institute, Dr. H. PROPACH, was kind enough to supplement my experimental material by sending me seed of some *Solanum* species.

On the *Solanum* material thus obtained I have made some investigations mainly concerning the conditions of polyploidy, sterility and incompatibility within the section *Tuberarium*. It is frankly admitted that in several cases these investigations would have been much improved by a more profound treatment of certain details. Unfortunately, this has sometimes been impossible owing to the dying off of the experimental plants, which under our climatic conditions are rather difficult to cultivate successfully. On the whole, however, the investigations have been planned on a fairly wide basis.

The study of polyploidy takes in both primary and secondary polyploidy. Comparative, mainly meiotical investigations have been performed on types representing various kinds and degrees of ploidy. Here, the studies on artificial tetraploids and octoploids may be mentioned in the first place.

The problem of secondary polyploidy has been approached by studies of secondary association and secondary balance.

The causes of sterility have mainly been attached from the cytological point of view. The correlation between pollen sterility and chromosomal aberrations has been studied in populations of diploid species. The effects of chromosomal variation on fertility have been investigated in progenies of crosses between species representing different degrees of ploidy and in the artificial polyploids mentioned above.

Finally, the causes of incompatibility have been investigated in some species crosses.

In connection with these investigations certain collateral problems, e. g. negative correlation of chiasma frequencies, have been studied.

Some of the results of these investigations have been published earlier (LAMM, 1938, 1941, 1943).

During the years 1933—1937 the plant material was cultivated at Svalöf, during 1938 at a private farm in the neighbourhood of Alnarp, and from 1939 onwards at The Horticultural Research Station at Alnarp, Sweden. I here wish to express my gratitude to the Swedish Seed Association and to the Horticultural Research Station at Alnarp for granting me the loan of experimental grounds and of manual help. This support was accorded me thanks to the interest and kindness shown in my investigations by the former and present heads of the Swedish Seed Association, Prof. H. NILSSON-EHLE and Prof. Å. ÅKERMAN, and by the former and present heads of the Horticultural Research Station of Alnarp, Prof. C. G. DAHL and Dr. F. NILSSON.

From the very beginning of my work I have enjoyed the benefit of Prof. A. MÜNTZING's valuable advice and wide experience. To Dr. Å. GUSTAFSSON and Dr. A. LEVAN I wish to tender my sincere thanks for encouragement and advice and for the great interest they have shown in my investigations.

I am very greatly indebted to Dr. O. TEDIN for valuable advice concerning the statistical treatment, and to Dr. G. TOMETORP for his critical scrutiny of the manuscript.

To many persons for valuable assistance in various ways, including discussions, etc. I am also indebted and especially I wish to express

my thanks here to Miss MAGNA PALM, Svalöf, for valuable technical assistance.

Finally, I desire to mention that the accomplishment of the work was to a great extent rendered possible and facilitated by the valuable and untiring help given me by my wife, Mrs. INGA LAMM.

## II. EXPERIMENTAL.

### 1. MATERIAL AND METHODS.

#### A. TAXONOMY.

The genus *Solanum* L. is divided by taxonomists into several sections or subgenera (DUNAL, 1852; BITTER, 1912/13). One of these, *Pachystemonum* DUN., is further divided into five subsections, of which the subsection *Tuberarium* DUN. comprises the tuber- or at least the stolon-producing *Solanum* species. All the species of the present investigation belong to this subsection (in this paper called the section *Tuberarium*).

The section *Tuberarium* has in its turn been further divided into subsections and groups with respect to the position of the articulation of the pedicel, shape of the corolla, division of the leaf, and other characteristics (BITTER, 1912/13; WITTMACK, 1914; RYDBERG, 1924). Recently taxonomists for all the Russian scientists of the VAVILOV school have furnished contributions to the taxonomy of this section (BUKASOV, 1930, 1933, 1937, 1938, 1940; JUZEPCZUK and BUKASOV, 1929; JUZEPCZUK, 1937).

By their extensive work a great number of new species have been investigated and described, and species established by earlier taxonomists have been subdivided into new »species». In some cases, however, it seems rather doubtful whether they deserve the rank of species (STELZNER and LEHMANN, 1939, p. 101). Indeed, the differentiation of the section *Tuberarium* into subordinate groups has probably gone too far. Nevertheless, in this paper, the Russian classification has been adopted as being at present the best available.

The taxonomical relations between groups and species have been illustrated by various schemes (SIDOROV, 1937; BUKASOV, 1937, 1938). To facilitate reference the scheme of SIDOROV (1937) has been given below (Table 1), but the number of species has here been restricted to comprise mainly those which in one or another connection are mentioned in this paper. It must also be pointed out that there is considerable disagreement between the different schemes suggested by the Russian taxonomists. Future investigations will probably necessitate a more or

less radical revision of the relations suggested by earlier and present taxonomists.

TABLE 1. *Scheme of taxonomic relations within the section Tuberarium* (*after* SIDOROV, *1937*).

(Only species mentioned in this paper are listed below.)

| Group | Species (* = cultivated) | 2n | Geographical distribution |
|---|---|---|---|
| Stellata | | | |
| Pinnatisecta | | | |
| Commersoniana | *S. Henryi* BUK. et LECHN. | 24 | Uruguay |
| | *S. Commersonii* DUN. | 36 | » |
| | *S. chacoense* BITT. | 24 | Argentine, Paraguay |
| | *S. Milanii* BUK. | 36 | » |
| Jamesiana | *S. Jamesii* TORR. | 24 | Mexico |
| Cardiophylla | *S. cardiophyllum* LINDL. | » | » |
| Bulbocastana | *S. bulbocastanum* DUN. | » | » |
| Rotata | | | |
| Articulata | | | |
| Oxycarpa | *S. colombianum* DUN. | 48 | Colombia |
| Tuberosa | | | |
| Polyadenia | *S. polyadenium* GREENM. | 24 | Mexico |
| Vaviloviana | *S. Vavilovii* JUZ. et BUK. | » | Peru |
| Aracciana | *S. aracc-papa* JUZ. | » | » |
| | *S. Bukasovii* JUZ. | » | » |
| Andigena | *S. Rybinii* JUZ. et BUK.* | » | Colombia |
| | *S. stenotomum* JUZ. et BUK.* | » | Bolivia, Peru |
| | *S. phureja* JUZ. et BUK.* | » | Bolivia |
| | *S. chaucha* JUZ. et BUK.* | 36 | Bolivia, Peru |
| | *S. andigenum* JUZ. et BUK.* | 48 | Bolivia, Peru, Colombia, Ecuador, Argentine, Mexico |
| | *S. ajanhuiri* JUZ. et BUK.* | 24 | Bolivia |
| Trigonohypsa | *S. Juzepczukii* BUK.* | 36 | Bolivia, Peru |
| | *S. curtilobum* JUZ. et BUK.* | 60 | » » |
| Eutuberosa | *S. tuberosum* L.* | 48 | Chili |
| Borealia | *S. Fendleri* A. GRAY | » | Mexico |
| Longipedicellata | *S. Antipoviczii* BUK. | » | » |
| | *S. ajuscoense* BUK. | » | » |
| Demissa | *S. verrucosum* SCHLECHTD. | 24 | » |
| | *S. demissum* LINDL. | 72 | » |
| Acaulia | *S. acaule* var. *subexinterruptum* BITT. | 48 | Bolivia |
| | *S. punae* JUZ. | » | Peru |

## B. ORIGIN AND CLASSIFICATION OF THE EXPERIMENTAL MATERIAL.

The origin of my own experimental material of *Solanum* species has been mentioned briefly in the introduction. This material comprised the following species:

*S. stenotomum* (JUZEPCZUK and BUKASOV, 1929) is a cultivated diploid polymorphic species. The Svalöf material of this species originated from the regions near the Lago Titicaca of Bolivia, where it had been collected under the name of Khati blanca by the late Professor E. NORDENSKIÖLD.

As the tuber formation was poor under the long-day conditions of Svalöf and the tubers were infected by virus (cf. BUKASOV, 1933, p. 186), the species had to be propagated by seed. As a result of this a certain degree of inbreeding degeneration probably occurred. Most diploid *Solanum* species are allogamous, and PROPACH (1940) points out that when material is collected in foreign countries seed should be preferred to tubers. The flower colour of the original clones was light violet. In their sexual progenies plants with white and violet-blue flowers occurred, but other characters such as leaf lobulation also segregated.

Samples from my herbarium collection were sent to Dr. H. PROPACH, Kaiser Wilhelm-Institut für Züchtungsforschung, Müncheberg (Mark), for comparison with the Institute's collection of South American potatoes. In a letter Dr. PROPACH writes: »Ihr *S. stenotomum* gehört zweifellos zu dieser Art. Ich selbst habe im Augenblick nur noch die Form *violaceum* lebend, die nur durch violette Farbe der Knollenschale abweicht. In den anderen Merkmalen ist aber so völlige Übereinstimmung, dass über die Zugehörigkeit kein Zweifel sein kann; mein Material stammt übrigens von BUKASOV».

*S. Rybinii* (JUZEPCZUK and BUKASOV, 1929; BUKASOV, 1930) is a cultivated diploid Colombian mountain species. Seed of this species was obtained in 1938 from Dr. H. PROPACH. It possesses the property of sprouting within a few weeks from harvesting the tubers (Imperial Bureau, 1936). In breeding for earliness attempts to utilize *S. Rybinii* are being made (BUKASOV, 1936). There is some susceptibility to *Phytophtora* (blight) (BUKASOV, 1933, p. 187). This species is self-sterile (RYBIN, 1940). The tuber formation is satisfactory even under long-day conditions (BUKASOV, 1937). The tubers were red and the flowers a deep reddish-violet. The seed obtained, however, segregated, and plants with white flowers occurred.

*S. verrucosum* SCHLECHTD. (BITTER 1912/13) is a wild diploid Mexican species of the group *Demissa*. It is autogamous (PROPACH, 1940) and exceedingly fertile. It only gives out tubers under short-day conditions (STELZNER and TORKA, 1940). Like most Mexican potato species, it is characterized by blue-violet flowers. Of this species, too, I obtained seed from Dr. H. PROPACH and it bred true.

The species is resistant to blight (BUKASOV, 1936).

*S. chaucha* (JUZEPCZUK and BUKASOV, 1929) is a cultivated triploid species, which is related to the diploid *S. phureja*. Like this species it is early (BUKASOV, 1938). The Svalöf specimens of this species had the same geographical origin as *S. stenotomum*.

Samples of *S. chaucha* from my herbarium collection were sent to Dr. H. PROPACH for comparison with his Institute's species collection. From Dr. PROPACH I obtained the following report: »Unsere beiden Formen von *S. chaucha* sind in der Knollenform beide anders als Ihre, sie haben gedrungenere Knollen. In der Schalenfarbe weicht eine ab. Die Chromosomenzahl unserer Klone habe ich noch nicht untersucht, weil ich bisher keine Kombinationen mit dieser Art gemacht habe; die Russen geben ja auch $2n = 36$ an. Die Exsiccate unseres Materials weichen aber sonst nicht von Ihrem Exemplar ab, so dass Sie Ihren Klon mit gutem Gewissen als *S. chaucha* bezeichnen können».

The tubers of the Svalöf clones were elongated oval and their skin was made up of red-violet and colourless areas. This type of tubers is also characteristic of *S. phureja* (JUZEPCZUK and BUKASOV, 1929). The preservation of this material was entirely dependent on vegetative reproduction by tubers. The tuber formation was poor, and the plants were infected by blight and virus (cf. BUKASOV, 1933, p. 187). BUKASOV (1937) has found that triploid species soon die off when cultivated in Europe.

*S. tuberosum* L., the common potato species cultivated in Europe, is tetraploid (ELLISON, 1935, pp. 1 and 24; LUNDEN, 1937, p. 4).

The origin of this species remains obscure. The first introduction of this or a related species into Europe probably took place from the Peru—Bolivian centre. There is a record of a present of potatoes being sent to Philip the Second of Spain in 1565 from Cuzco, Peru (Imp. Bureau, 1936). MACMILLIAN (1940) also thinks that *S. tuberosum* originates from that part of South America, while Russian taxonomists (JUZEPCZUK and BUKASOV, 1929; JUZEPCZUK, 1937) consider the Chilean centre to be the home of this species. Round about 1580 the species was again introduced into Europe, this time by seafarers from Chile (Imp. Bureau, 1936), and it is possible that this second introduction has been of more importance to the creation of European potato-varieties than the first one.

In my own investigation I have mainly worked with four varieties of *S. tuberosum*, viz. Deodara, Pepo, Gammal röd (Old red) and 1935—33062 (a number assigned by the Potato Department of the Swedish Seed Association, Svalöf, to a variety selected from the progeny of the cross Greta × Fürstenkrone. By me this variety has been called 36/209). These varieties were chosen for their good seed-producing properties.

*S. andigenum* (JUZEPCZUK and BUKASOV, 1929) is the most extensively grown potato in all the countries bordering on the Andes. Cultivated and wild forms are known. It seems to be closely allied to

*S. tuberosum*, from which it is in many respects indistinguishable (Imp. Bureau, 1936). The main differences between these species have been described by BUKASOV (1937). Here it will only be pointed out that the formation of tubers in *S. andigenum* decreases under long-day conditions. Of this species a number of samples representing different varieties were collected by Dr. HAMMARLUND. One of these samples (Field No. 36/213) originating from Sacambaya, Cercado, Bolivia, was chosen by the author for experimental work because of its relatively satisfactory tuber formation and abundant seed setting. The tubers of this variety were elongated oval with a colourless skin, the flowers were light violet.

*S. acaule* BITT. is a wild tetraploid species, a native of the mountain regions of Bolivia, Peru and Argentine. It is found at altitudes occasionally reaching 5000 metres and penetrates up to the very limit of eternal snows. Both its haulm and flowers remain uninjured by temperatures of — 8° C (Imp. Bureau, 1936). Because of this valuable property it is of great interest to practical plant-breeding. It is unfortunately difficult to cross with other species. Morphologically it differs very much from other potato species (see Fig. 68). The flowers are a pale blue-violet.

In the *Acaulia* (see Table 1) the scarcely visible articulation of the pedicel is situated immediately beneath the calyx, and there is no connection between the anthers. According to BUKASOV (1937), this is the youngest group within the section *Tuberarium*.

*S. acaule* is self-fertile and gives plenty of seed (PROPACH, 1938 a; STELZNER, 1943 b). Tubers are only formed under the conditions of short day (STELZNER and TORKA, 1940).

From the Potato Department of the Swedish Seed Association, Svalöf, I obtained seed of this species harvested from progenies of plants originating from Bolivia, where this species had been collected by Dr. HAMMARLUND.

Through the kind assistance of Dr. E. HULTÉN, Lund, this species was found to correspond to *S. acaule* var. *subexinterruptum* BITT. (BITTER, 1912, XI; p. 393). I should here mention that in an earlier paper I unfortunately referred to this species under the name of *S. punae* JUZ. (LAMM, 1943). By JUZEPCZUK (1937) *S. acaule* has been divided into one Peruvian and two Argentinian species. The former, *S. punae*, according to JUZEPCZUK (1937), is possibly a form of the var. *subexinterruptum* BITT. In *S. punae*, however, there are about three segments (2—4) in the odd pinnately-divided leaf. The pedicels are rather hairy and the fruit is violet (JUZEPCZUK, 1937), while in *S. acaule* var. *subexinterruptum* BITT. the

number of leaf segments is about five (3—6), the pedicels are subglabrous and the fruit is green.

*S. curtilobum* (JUZEPCZUK and BUKASOV, 1929) is a cultivated pentaploid montane species native to Bolivia and Peru. Although it belongs to the *Articulata* (see Table 1), it is probably related to the *Acaulia* (JUZEPCZUK, 1937). It is frost-resistant (BUKASOV, 1933), though not to the same degree as *S. acaule*. Like this last-mentioned species it is rather susceptible to blight (BUKASOV, 1933; STELZNER and LEHMANN, 1939). Tuber formation under long-day conditions is poor (BUKASOV, 1933, p. 186), but clones are known which do produce tubers under such conditions (HACKBARTH, 1935).

The Svalöf material of this species which I selected for experimental work had been collected under the name of Luki Choquepitu by Dr. HAMMARLUND at Tuaku, Bolivia.

The tubers were small, flat-round, and had an uncoloured skin. The tuber formation under the long-day conditions of Svalöf was poor. The flowers were blue-violet and characterized by the rather short lobes of the calyx. After some years of vegetative propagation my clones of this species died off.

## C. TECHNICAL METHODS.

### a. Cytological methods.

It was often difficult to get good fixations of the somatic chromosomes in *Solanum*. The chromosomes had a tendency to stick together, which could be counteracted by a mild, cold treatment about twenty-four hours before fixation. The effects produced by more intense cold treatment will be discussed later. Each plant was separately planted in a small pot and grown in the greenhouse. Root-tips were not taken until the plants had become well established.

For fixations of somatic chromosomes I used as a rule MÜNTZING's modification of NAVASHIN's solution (MÜNTZING, 1932). Sometimes very good results were obtained by using Medium Flemming (LA COUR, 1937). LEWITSKY's (1934) Platinic Formalin (weak), which has been recommended by SEPELEVA (1937), and BENDA's Fixative recommended by MEURMAN and RANCKEN (1932) and ELLISON (1935) were also tried but with less success. After being embedded in paraffin wax the root-tips were cut into sections 14 $\mu$ thick and stained with gentian violet. As far as possible two or three plates of each plant were counted.

For investigations of the meiotic divisions in the embryo-sac mother-cells (EMC's) the ovules were first treated with CARNOY for a few minutes and then fixed in MÜNTZING's modification of NAVASHIN's solution. The sectioning and staining of such slides have been described by LAMM (1937).

Excellent slides of the meiotic divisions of the pollen mother-cells were obtained by the smear method. These slides were fixed in the NAVASHIN solution mentioned above, but diluted with an equal quantity of water. Sometimes it was

found favourable to dilute the solution still more. These slides were stained in gentian violet.

For the purpose of investigating the tapetum cells the anthers were fixed in the same solution as the root-tips, embedded in paraffin wax and cut. The meiotic divisions in the pollen mother-cells of such slides were never fixed so well as in the successful smears.

In some cases nucleoli were studied in slides stained with FEULGEN and BHADURI (SEMMENS and BHADURI, 1939).

The drawings were prepared with the aid of a camera lucida.

The fertility of pollen from mature anthers was studied in aceto-carmine glycerine (1 : 1). In each slide prepared for such a determination about 200 grains were counted. Grains full of stained material were counted as »good», grains irregular in outline, imperfectly filled, or empty, were counted as »poor». In this paper pollen fertility refers to the percentage of »good» grains.

The diameter of pollen-grains and the length of stomata were determined in these aceto-carmine glycerine slides. In the former cases only »good» pollen-grains were measured, these being then chosen at random for measurement. In the latter case the stomata were chosen at random from those within the slide. Slides were, however, prepared of the epidermis of big leaves and leaves of medium size. From these leaves epidermis was taken from the underside of the principal leaf segment No. 2 counted from the apical end of the rachis of the odd pinnately-divided leaf. (Other measurements, such as the thickness, length and breadth of the leaves, were also made on the left member of the same pair of leaflets.)

## b. The technique of crossing and grafting.

The technique of crossing has on the whole been that described by STELZNER and LEHMANN (1939, pp. 106—108). The crosses, however, were performed in the mornings, although the afternoons would perhaps have given better results. Protection by bagging was not used at all, as pollination may suffer from the often unfavourable atmospheric conditions inside the bags. Necessary adequate protection was secured simply by isolating the female partner in a greenhouse.

From several crosses it was rather difficult to obtain successful results. *A priori* this could be expected, since the crosses were interspecific and the partners often had different chromosome numbers. In some cases the difficulties were confined to one direction of crossing, e. g. *S. tuberosum* × *S. curtilobum* (see p. 82).

Successful crossing depends very largely on various climatic conditions, such as temperature, humidity, time, and intensity of illumination (STOUT and CLARK, 1924; STEVENSON and CLARK, 1937; PROPACH, 1938 b; CLARKE and LOMBARD, 1939; STELZNER and LEHMANN, 1939; PERLOVA, 1940). For example, back-crosses between some $F_1$ hybrids of *S. tuberosum* × *S. curtilobum* and their mother were tried on a large scale without success during six seasons. In the summer of 1943, however, these crossings succeeded, probably owing to favourable climatic conditions.

In some cases the crossing of ordinary plants failed, e. g. *S. chaucha* × *S. tuberosum* and *S. tuberosum* × *S. curtilobum*. When the female partner of these combinations was grafted on the tomato, the crosses succeeded without difficulty. Etiolated shoots from the tubers, or in the case of *S. acaule* the apical ends of the stolons, were cleft-grafted on young tomato plants of the variety »Danish export». As a rule two

leaves were left on the tomato plants (Fig. 68). The technique of grafting has been described by JØRGENSEN and CRANE (1927) and JØRGENSEN (1928 b).

STOUT and CLARK (1924) have discussed the relation between vegetative and sexual reproduction. Ever since T. A. KNIGHT at the beginning of the nineteenth century showed that the formation of berries in some early potato varieties was increased by prevention of tuber formation, a negative correlation between these kinds of reproduction has been assumed. Using the grafting method modern American scientists, however, consider they have shown that such a correlation does not exist, at least not within species. On the other hand, JURKOV (1936) claims that grafting has caused the development of fruits in potato varieties which are usually sterile, and that even difficult crosses have been rather successful. Besides tomato, other species of the family Solanaceae have been tried with more or less satisfactory results. Other methods, e. g. strangulation, have also proved successful in increasing the formation of berries (STELZNER and LEHMANN, 1939, p. 105).

When applying the method of grafting in my own experimental work, the grafted and normal plants were mostly cultivated under different conditions, e. g. greenhouse and field. Consequently those experiments cannot be used for the study of the effect of grafting on the success of crossing. In a few cases, however, grafted and normal plants were cultivated together under comparable conditions. I then found that the variety »Deodara» of *S. tuberosum* was a suitable scion, while the variety »Pepo» of the same species when grafted on the tomato dropped its buds. The effect of grafting on the pollen fertility of »Deodara» and of *S. acaule* will be discussed later.

In apple and pear trees the stock has some influence on the chemical and physiological properties of the scion, such as the sugar content and osmotic pressure (BEYME, 1937). It is a ready assumption that in certain cases an interaction of this description in grafted *Solanum* plants may favour the realization of interspecific crossing. In *Petunia nyctaginiflora* GYÖRFFY (1941) found that in heteroplastic graftings (2n grafted on 4n or 4n grafted on 2n) the somatic pressure of the scion was always higher than that of the stock.

## c. Induction of polyploidy by colchicine treatment.

Previous workers have induced tetraploidy or octoploidy in a number of *Solanum* species by colchicine treatment. Various methods of application have been practised (MÜNTZING and RUNQUIST, 1939; JOHNSTONE, 1939; LIVERMORE and JOHNSTONE, 1940; RYBIN, 1940; STELZNER, 1941). The induction of octoploidy in the tetraploid *S. acaule* has been described by LAMM (1943).

In the diploid species *S. Rybinii* and *S. verrucosum* and in the diploid $F_1$ hybrids between these species the present writer has induced tetraploidy by colchicine treatment of the seed. Two methods were employed.

In the first of these methods seed was placed in Petri dishes on filter-paper moistened with a tap water solution of 0,5 % colchicine. The treatment lasted for five days. The effect of this treatment was nearly lethal, but in 1941 one autotetraploid plant of *S. Rybinii* was obtained, and in 1943 one tetraploid plant of *S. verrucosum* and two tetraploid plants of the $F_1$ hybrids were obtained. A colchicine concentration of 0,4 % would probably have given better results (RYBIN, 1940; STELZNER, 1941).

The second method was more successful. In 1943 six autotetraploid plants of *S. Rybinii* were obtained as a result of treating fifty seeds. The dry seed was placed in a Petri dish on the surface of a colchicine agar jelly. This was made by mixing 2 parts of a 2 % colchicine with 1 part of 3 % agar solution in destilled water. After two days, just when the root-tips appeared, the seed was transferred to a pot with a seed compost suitable for the sowing and pricking of small seed plants.

### D. SYMBOLS REFERRING TO THE STATISTICAL AND CYTOLOGICAL INVESTIGATIONS.

Statistical methods have been employed on several occasions in the treatment of the material. The significance of the values found has been tested according to the methods of SNEDECOR (1938) and BONNIER and TEDIN (1940). To mark the probability (P) values in cases where the table of »t», of chi-square ($\chi^2$), or of variance ratio is used the significance has been given with asterisks as exponents in the following manner:

* $P = 0{,}05—0{,}01$
** $P = 0{,}01—0{,}001$
*** $P < 0{,}001$

When statistical symbols are used in this paper they are in accordance with the index of symbols given by SNEDECOR (1938, p. 381). It may be mentioned that for the average value the symbol M is used, for the number of variants the symbol n, and for degrees of freedom the symbol Df.

The following cytological symbols have been used. First metaphase = I—M, second metaphase = II—M, first anaphase = I—A, second anaphase = II—A, first telophase = I—T, second telophase = II—T, satellited chromosomes = SAT-chromosomes. Other abbreviations are in accordance with DARLINGTON and LA COUR (1942, p. 129).

## 2. INVESTIGATIONS ON DIPLOID SPECIES AND HYBRIDS.

### A. POLLEN FERTILITY.

During my earliest studies of the potato I noticed that partial pollen sterility was common in the diploid species. The pollen sterility of *S. tuberosum* had been the object of several investigations (see p. 110). Still, the causes of pollen sterility in this tetraploid species had not been satisfactorily explained. It seemed to me that corresponding investigations on diploid species would probably yield valuable results, facilitating the understanding of the more complicated conditions among tetraploids.

In 1937 I decided to study the correlation between pollen fertility and the meiotic behaviour of the diploid *S. stenotomum*. As my material of this species died off during the following years, these investigations were later carried on with *S. Rybinii* and *S. verrucosum*. The general meiotic behaviour of these species has also been studied.

These three diploid species are of widely different geographical origin. According to the taxonomist the autogamous *S. verrucosum* is more remotely related to the two other species, which are allogamous, than to each other (see Table 1).

When starting these investigations in 1937 I had 16 different clones of *S. stenotomum*, three of which did not flower. These clones originated from seed harvested in 1934 from three different plants of a progeny of sister-plants, which were grown together in the field. The seed was a result of free flowering, but as opportunity was at hand for either cross-pollination with sister-plants or self-pollination, the progenies must have been at least slightly inbred. The sister-plants in their turn originated from the free flowering of *S. stenotomum*, field No. 33/5—10, which was grown among clones of plants of the same species collected in Bolivia.

From the seed harvested in 1934 75 plants were raised the following year. Of these only 30 were tuber-forming. In 1937 the number of clones, as mentioned before, had been reduced to 16. This was the result of natural selection for attacks of especially virus and blight.

A population of *S. Rybinii* was raised in 1939 and a vigorous clone, field No. 39/8, was selected. From this, after controlled self-pollinations, seed was obtained in 1940 and 1942. The seed was used for the raising of 60 ordinary diploid plants and for colchicine treatment. Of these diploids, 20 were grown in 1941 and 40 in 1943.

A clone of *S. verrucosum*, field No. 41/9, was selected in 1941. Also here seed was obtained after controlled self-pollination. The seed was used in 1943 for the raising of ten ordinary diploid plants and for colchicine treatment.

With the expectation of eventually obtaining structural hybrids the cross *S. Rybinii* (39/8) $\times$ *S. verrucosum* (41/9) was performed in 1942. The crossing was successful, and plenty of seed was obtained. The seed was used in 1943 for the raising of 15 diploid $F_1$ hybrids and for colchicine treatment. These $F_1$ hybrids were characterized by blue-violet flowers and tubers. The blue-violet colour did not occur in *S. Rybinii*, but characterized the flowers of *S. verrucosum* (see p. 5). In contrast to this species but in accordance with their mother, *S. Rybinii*, 39/8, the $F_1$ hybrids formed tubers under the conditions of the long day. These facts proved that they must have been true hybrids.

Owing to lack of space in the open frames, where the plants were cultivated during 1940—1943, I had to restrict the number of plants

as far as possible. Hence the low number of plants in some of the progenies.

The pollen fertility of the ancestors and the diploid progenies is given in Table 2. Nearly all the plants are more or less pollen-sterile. This is also the case as regards the autogamous species *S. verrucosum*. To a certain degree this sterility might be due to external influences, such as unfavourable climatic conditions. Such factors could, however, hardly explain the rather poor pollen fertility of some of the plants in comparison with the high pollen fertility of others. Other factors

TABLE 2. *Pollen fertility in some diploid Solanum plants.*

| Category | Year | Total male sterile | Percentage of good pollen | | | | | | No. of plants |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 20 | 40 | 60 | 80 | 100 | |
| Ancestors (population) | | | | | | | | | |
| *S. stenotomum*, 33/5—10 ..... | 1933 | | | | | | 1 | | 1 |
| » *Rybinii*, 39/8 ..... | 1943 | | | | | | 1 | | 1 |
| » *verrucosum*, 41/9 ..... | » | | | | | | | 1 | 1 |
| Descendants | | | | | | | | | |
| *S. stenotomum* I* ..... | 1937 | 1 | 1 | 2 | 2 | 4 | 3 | 13 | |
| » *Rybinii* $I_1$ ..... | 1941 | 1 | 3 | 6 | 3 | 2 | 5 | 20 | |
| » » » ..... | 1943 | 2 | 3 | 3 | 6 | 12 | 14 | 40 | |
| » *verrucosum* ..... | 1943 | | 1 | 1 | | 2 | 6 | 10 | |
| » *Ryb.*, 39/8 × *S. verr.* 41/9, $F_1$ | 1943 | | | 1 | 2 | 5 | 7 | 15 | |

such as the action of genes and chromosomal aberration might also be responsible for the sterility. In order to investigate whether disturbances of the kind last mentioned are present in diploid *Solanum*, slides of meiosis were made of some plants representing various degrees of sterility. The result of these investigations will be described in the cytological section of this Chapter.

## B. CYTOLOGY.

### a. Somatic chromosomes.

The morphology of the chromosomes in some diploid *Solanum* species of the section *Tuberarium* has been studied by ŠEPELEVA (1937). These species had the same types of chromosomes, but the number of chromosomes of each type differed in the various species, and some of the types were entirely lacking in some species. These investigations

* The progeny was slightly inbred.

seem to be restricted to one plate of each species and have been criticized by PROPACH (1937 c) as not being altogether reliable.

Drawings of mitotic metaphases of root-tips of the diploid species studied by the author are shown in Figs. 1—4. The chromosomes are small and difficult to investigate. The root-tips have been fixed in modified NAVASHIN's solution. Figs. 2 and 3 refer to two plants of *S. Rybinii*, field Nos. 39/8 and 43/8—34. In the latter case the plant was kept for 48 hours before fixation in a cellar under a temperature of about 10° C. In the former case root-tips were taken direct from the plant cultivated in a greenhouse. There is a considerable difference in the contraction of the chromosomes of these two plants. Such differences in chromosome contraction are sometimes found between adjacent cells of the same root-tip, and contribute to the difficulties of chromosome morphological studies.

Figs. 1—4. Somatic metaphase plates of diploid *Solanum* plants. — Fig. 1, *S. stenotomum*; Figs. 2—3, S. *Rybinii*, normal, and of a root-tip that has been slightly cooled before fixation; Fig. 4, *S. verrucosum*. The chromosome complements contain two SAT-chromosomes. — × 2550.

In the diploid species studied by me, at least three individual chromosome pairs can be distinguished from the rest of the complement. One of these has relatively large trabants attached terminally to the short arm. In some diploid species ŠEPELEVA has found more than one pair of such SAT-chromosomes. The other two pairs without trabants are longer and have a secondary constriction in the long arm (Type *a* of ŠEPELEVA). As for the rest of the complement, also here differences in size and structure can be seen. Thus there seems to be another pair of chromosomes with a secondary constriction in the long arm. There is at least one pair of rather short equal-armed chromosomes (Type *c* of ŠEPELEVA), and there are chromosomes with median, submedian and subterminal constrictions.

It is possible that differences of the kind described by ŠEPELEVA exist. With the cytological technique applied by me, however, it has

not been possible to prove differences between the idiograms of *S. stenotomum*, *S. Rybinii* and *S. verrucosum*.

After mild cold treatment of the somatic chromosomes I have noticed that the distal ends of the short arms of chromosomes with secondary constrictions seem to contract less than the rest of the chromosome. These ends are probably heterochromatic. In *Nicotiana glauca* × *N. Langsdorffii* the distal ends of some chromosomes are thought by KOSTOFF (1939) to be heterochromatic.

b. Chromosomal aberration and pollen fertility.

Good slides suitable for meiotical studies were obtained of seventeen different diploids from the plants listed in Table 2. These plants are enumerated below. In some of them quite obvious chromosomal aberrations occurred, e. g. inversion, segmental interchange, and fragmentation. These plants will be referred to as »aberrant» in contradistinction to the »normal» plants, although strictly speaking this classification is not adequate.

*List of diploid plants meiotically investigated.*

| Species | Field number | Pollen fertility, % | Classification of meiosis | Remarks |
|---|---|---|---|---|
| *S. stenotomum* . . . . | 37/114 | 11 | aberrant | slightly inbred |
| | » /120 | 30 | normal | » » |
| | » /131 | 93 | » | » » |
| | » /133 | 0 | aberrant | » » |
| | » /134 | 47 | normal | » » |
| | » /137 | 73 | » | » » |
| *S. Rybinii* . . . . . | 39/8 | 62 | » | parental clone |
| | 41/8—1a | 0 | aberrant | inbred ($I_1$) |
| | 43/8—28 | 74 | normal | » » |
| | » —32 | 16 | aberrant | » » |
| | » —34 | 88 | normal | » » |
| | » —35 | 31 | » | » » |
| | » —36 | 39 | » | » » |
| *S. verrucosum* . . . . | 41/9 | 92 | » | parental clone |
| | 43/9—10 | 39 | aberrant | selfed progeny |
| *S. Ryb.* × *S. verr.* . . . | $43/8 \times 9_7$ | 78 | normal | $F_1$ hybrid |
| | » $/8 \times 9_{18}$ | 82 | » | » » |

The details of the meiotical investigations on these seventeen plants will be reported in the following sections of this Chapter.

It is interesting to note that the two male-sterile plants as well as most of the plants having a high degree of partial pollen sterility were

also characterized by chromosomal aberration. In the latter category exceptions, however, occur, e. g. *S. stenotomum*, 37/120. This indicates a possible causal connection between sterility and chromosomal disturbances.

In *S. verrucosum*, which contrary to the other species is autogamous, two plants out of ten showed a high degree of partial pollen sterility (see Table 2). One of these plants, field No. 43/9—10, was cytologically examined, and proved to be aberrant. The other plant deviated by its pale blue-violet flowers from the parental clone, while all the other nine plants had the same flower colour as the parental clone. Unfortunately this plant was not investigated with a view to seeing whether it was also cytologically aberrant.

The relation between these observations in *Solanum* and similar but more comprehensive earlier investigations of the same kind in other species (MÜNTZING, 1939; MÜNTZING and PRAKKEN, 1941) will be discussed later.

c. General meiotic behaviour of normal plants.

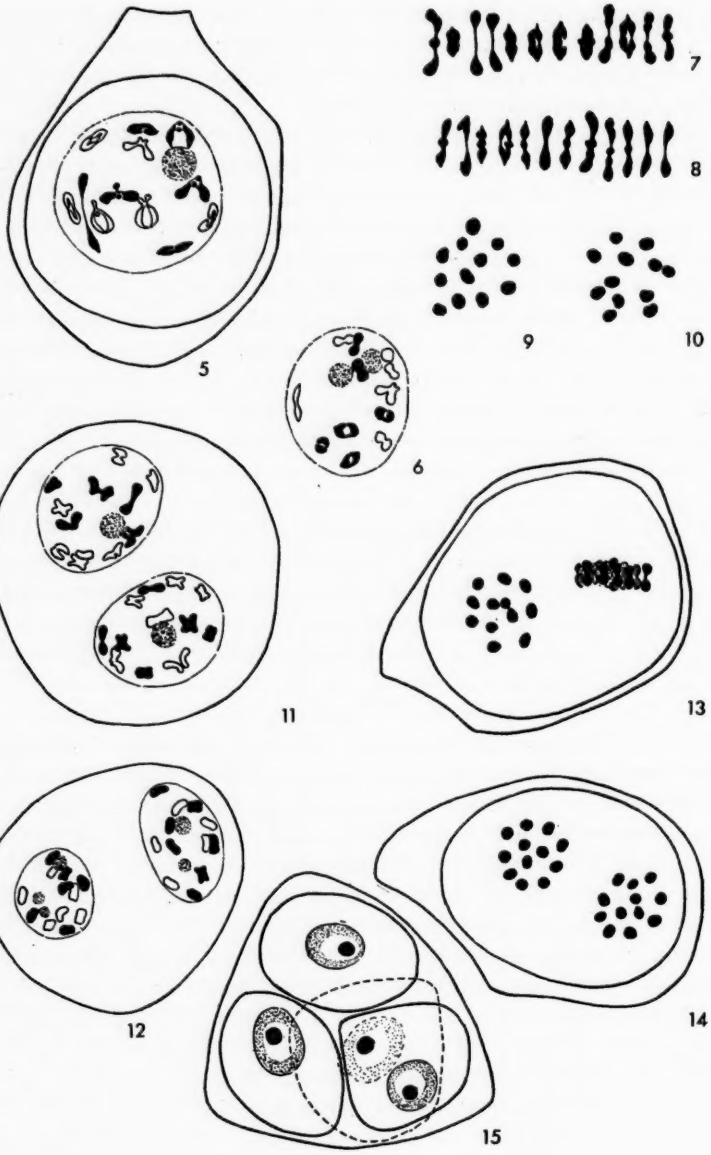
Meiosis in a diploid representant of the section *Tuberarium* has been described by MÜNTZING (1933 a). This diploid probably belonged to the species *S. stenotomum*. Thanks to the improved technique of fixation, I have been able to undertake a close study of the diploid meiosis in the PMC's.

Early stages of prophase have not been examined. The material is not suitable for fixation at these stages, at any rate not with the fixatives used by me. Good slides have been obtained of diakinesis and the successive stages. Fig. 5 shows early diakinesis in *S. Rybinii*, 43/8—28. The chromosomes are united by one or two chiasmata. These may be terminal, subterminal or interstitial. The case was similar with other plants of the same species, as also with plants of *S. stenotomum*, *S. verrucosum* and the $F_1$ hybrid *S. Rybinii* $\times$ *S. verrucosum*. MÜNTZING (1933 a) in his diploid *Solanum* and UPCOTT (1935) in *Lycopersicum esculentum* found that almost all the chiasmata were terminalized at diakinesis. In *S. melongena* JANAKI AMMAL (1934) found that there was less terminalization than in *Lycopersicum* and *Datura*. One bivalent is as a rule associated with the nucleolus. This bivalent probably consists of the SAT-chromosomes. Occasionally this bivalent was situated between two large nucleoli, and in contact with both of them. Fig. 6 illustrates this situation in a PMC nucleus of *S. stenotomum*, 37/131, at a late diakinesis. A similar configuration has been described by

HÅKANSSON (1943) in a hybrid of *Godetia*. Twin nucleoli of this kind have also been observed in diploid *Lycopersicum esculentum* (LESLEY and LESLEY, 1935) at early prophase stages, but not at diakinesis. At pro-metaphase the nucleolus disappears.

In Figs. 7—8 two metaphases of the first division in side view are illustrated. These metaphases originate from the $F_1$ hybrids $43/8 \times 9_7$ and $43/8 \times 9_{13}$. In Fig. 7 the number of chiasmata amounts to 18, 14 of which are terminal. In Fig. 8 the corresponding numbers are 13 and 5. The non-hybrid plants also produced the same types of gemini, as can be seen from the bottom lines of Table 3. Figs. 9—10 show polar views of the I—M of *S. stenotomum*, 37/131. In the first of these plates there is no secondary association, while in the other plate eight of the bivalents are secondarily associated in groups of two bivalents each. Secondary association in *Solanum* and its bearing on secondary polyploidy will, however, be treated in a special Chapter.

In the normal plants there is a clean separation of the bivalents during I—A. At interphase the chromosomes in the daughter-nuclei may be easily distinguished and counted. Fig. 11 shows interphase in a PMC of *S. Rybinii*, 43/8—35. Most interphase nuclei have one nucleolus (Fig. 11), but in all the three diploid species investigated by the present writer nuclei with two or very exceptionally three nucleoli also occur. The latter situation is illustrated in Fig. 12, which shows a PMC of *S. stenotomum*, 37/137, at the stage of interphase. These slides were treated according to SEMMENS and BHADURI (1939), the nucleoli being stained a clear green. These additional nucleoli may be quite free from contact with any chromosome (see Fig. 12). In *Sesbania* JACOB (1941, p. 238) has shown that in some species not only the SAT-chromosomes but also secondarily constricted chromosomes produce nucleoli. In *Solanum*, besides SAT-chromosomes, secondarily constricted chromosomes occur (cf. p. 14), and probably some of these may form nucleoli. In *Nicotiana sylvestris*, in addition to the chromosomes with definite nucleolus-organizing bodies, there are other chromosomes sometimes associated with nucleoli (GOODSPEED and AVERY, 1939). Another possible explanation is that these additional nucleoli are remnants of the nucleolus formed on the SAT-chromosome. In *Pisum* HÅKANSSON and LEVAN (1942) have shown that the same pair of separating chromosomes may form more than the two expected nucleoli. In *Lycopersicum esculentum* the occurrence of two or three small nucleoli in each daughter-nucleolus during interphase has been reported by LESLEY and LESLEY (1935). Here, in addition to the pair of SAT-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chromosomes, there is only one pair of secondarily constricted chromosomes (UPCOTT, 1935). According to DARLINGTON (1937, p. 36), one of the pair of chromosomes in *Lycopersicum esculentum* is characterized by an intercalary trabant. Unfortunately, his observations on the chromosome morphology of the rest of the complement have not been published. Further studies on the behaviour and formation of nucleoli in *Solanum* are needed.

At II—M the sister plates of the PMC's are arranged in various ways in relation to each other. Fig. 13 of *S. stenotomum*, 37/131, shows the most common orientation. In the metaphase group seen in polar view there is one group of three secondarily associated chromosomes. Sometimes the homotypic spindles were parallel, as shown in Fig. 14 of the same plant. Here, there is only one group of two secondarily associated chromosomes in the right plate. As mentioned before, the phenomenon of secondary association will be discussed in a special Chapter. In normal plants dyads also are sometimes formed by the fusion of homotypic spindles (cf. MÜNTZING, 1933 a, p. 234).

After a clean II—A a tetrad is formed by wall formation. In each nucleus one to two and occasionally three nucleoli have then been counted (see Table 7). Fig. 15 shows a normal tetrad of *S. stenotomum*, 37/131.

### d. Chiasma formation of normal plants.

Table 3 gives the frequency of chiasma formation at I—M in 20 complete PMC nuclei from each of the nine different diploid plants which have been used for this study. The chiasma frequencies are expressed as the mean numbers of chiasmata per bivalent. The total terminalization coefficient is also given.

*Statistical.* — An analysis of variance of the primary figures of Table 3 is given on p. 20, below.

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Figs. 5—15. Meiosis in the PMC's of normal diploid *Solanum* plants. — Fig. 5, diakinesis of *S. Rybinii*. One bivalent is associated with the nucleolus; Fig. 6, an exceptional diakinesis nucleus of *S. stenotomum* with twin nucleoli; Figs. 7—8, I—M groups (separately drawn) from $F_1$ hybrids between *S. Rybinii* and *S. verrucosum* showing differences in X-frequency and terminalization; Figs. 9—10; I—M plates of *S. stenotomum* in polar view without and with secondary association of bivalents; Fig. 11, typical interphase of *S. Rybinii*. Each nucleus contains one nucleolus; Fig. 12, exceptional interphase in *S. stenotomum*. One of the nuclei contains two, one three nucleoli; Figs. 13—14, II—M groups of *S. stenotomum* with perpendicular and parallel orientation of the spindles. Fig. 13 shows a group of three secondarily associated chromosomes; Fig. 15, tetrad of *S. stenotomum*. — × 2550.

TABLE 3. *The types and frequencies of chromosome pairing at I—M in some diploid Solanum plants.*

| Pairing types No. of Xta per bivalent | | | Frequencies of PMC's of the different pairing types | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *S. Rybinii* | | | | | *S. Rybinii* × *S. verrucosum* | | *S. verrucosum* | *S. stenotomum* |
| 0 | 1 | 2 | 39/8 | 43/8 −28 | 43/8 −34 | 43/8 −35 | 43/8 −36 | 43/8 ×9₇ | 43/8 ×9₁₃ | 41/9 | 37/131 |
| | 12 | | | | | | | 1 | | | |
| | 11 | 1 | | | | | | 2 | 3 | | |
| 1 | 9 | 2 | | | | 3 | | 1 | | | |
| | 10 | 2 | | 1 | 1 | | | 4 | 2 | 7 | |
| 1 | 8 | 3 | 1 | | | | | | | | |
| 2 | 6 | 4 | | | | 1 | | | | | |
| | 9 | 3 | 2 | 2 | 5 | 3 | | | 2 | 7 | |
| 1 | 7 | 4 | 2 | | 1 | 1 | 1 | | | | |
| | 8 | 4 | 4 | 1 | 3 | 2 | 1 | 3 | 3 | 3 | |
| 1 | 6 | 5 | 1 | | 1 | 2 | 1 | 1 | 1 | | |
| | 7 | 5 | 3 | 2 | 2 | 2 | 1 | 1 | 3 | 3 | 1 |
| 1 | 5 | 6 | | | | | | 1 | | | |
| | 6 | 6 | 5 | 3 | 4 | | 3 | 4 | 3 | | 3 |
| 1 | 4 | 7 | | | | 2 | 1 | | | | |
| | 5 | 7 | 1 | 3 | 3 | 1 | 3 | 2 | 2 | | 4 |
| 1 | 3 | 8 | | | | | | | 1 | | 1 |
| | 4 | 8 | | 4 | | 3 | 3 | | | | 3 |
| | 3 | 9 | | 3 | | | 3 | | | | 1 |
| | 2 | 10 | 1 | 1 | | | 2 | | | | 6 |
| | | 12 | | | | | 1 | | | | 1 |
| Total No. of PMC's | | | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| No. of univalents . . | | | 8 | | 4 | 20 | 6 | 6 | 4 | | 2 |
| » » rod-bivalents | | | 136 | 100 | 145 | 134 | 90 | 159 | 153 | 178 | 75 |
| » » ring » | | | 100 | 130 | 93 | 96 | 147 | 78 | 85 | 62 | 164 |
| Mean X frequency | | | 1,40 | 1,54 | 1,38 | 1,36 | 1,60 | 1,31 | 1,35 | 1,26 | 1,68 |
| Terminaliz. coeff. | | | 0,87 | 0,90 | 0,88 | 0,90 | 0,92 | 0,84 | 0,78 | 0,88 | 0,91 |

*Analysis of variance referring to Table 3.*

| Variation of chiasma frequencies | Df | Sum of squares | Mean square |
|---|---|---|---|
| Inter-plant ..... | 8 | 38,9833 | 4,8729*** |
| Intra- » | | | |
| Inter-cell (error) ..... | 171 | 47,9333 | 0,2803 |
| Intra- » ..... | 1980 | 492,6667 | 0,2488 |
| Total | 2159 | 579,5833 | |

Minimum differences (D) between mean numbers of Xta per bivalent.

$D = t^{*} \quad (1{,}960) \times \sqrt{2} \times 1/\sqrt{240} \times \sqrt{0{,}2803} = 0{,}095^{*}$

$D = t^{**} \quad (2{,}576) \times$ » » » $= 0{,}125^{**}$

$D = t^{***} \quad (3{,}291) \times$ » » » $= 0{,}159^{***}$

The minimum differences for various degrees of significance between the chiasma frequencies have also been calculated. (Alternative methods are here possible). The thirty-six possible comparisons between chiasma frequencies have given the following results. The chiasma frequency of a slide of *S. stenotomum*, 37/131, is significantly higher than those in all other slides save that of *S. Rybinii*, 43/8—36. There are significant differences (**) between the two slides of the parental plants *S. Rybinii*, 39/8, and *S. verrucosum*, 41/9, but not between the slides of these plants and those of their two $F_1$ hybrids. The slides of $I_1$ (inbred for one generation) plants of *S. Rybinii*, 43/8—36 and 43/8—28, have significantly higher chiasma frequencies (*** and **) than the slide of their parent *S. Rybinii*, 39/8. As to the causes of these differences conclusions must be drawn with the greatest caution. In dealing with the chiasma frequency of an organism the environment must be taken into consideration (MATHER, 1935 a). In the *Solanum* species of the section *Tuberarium* these effects are probably rather marked (cf. p. 39). The plants listed in Table 3 were fixed on different days and partly also in different years. In *Triticum* MATHER (1935 a) found that the frequencies of chiasma formation varied from day to day, but there was no evidence of variation between plants fixed on the same day. The results of these investigations on chiasma frequency in *Solanum*, however, give suggestions for further work.

With respect to the results of Table 3 some rather likely conclusions may be drawn. One of these conclusions is that the chiasma frequency in the section *Tuberarium* has probably been underestimated by previous authors. MÜNTZING (1933 a, p. 225), in his investigation of diploid *Solanum*, did not observe more than one chiasma per bivalent. He concludes that the chiasma frequency in any case is very low. PROPACH (1938 a, p. 383) found an average chiasma frequency of 1,09 chiasmata per bivalent in the section *Tuberarium*. According to UPCOTT (1935), the chiasma frequency in diploid *Lycopersicum esculentum* was 1,66. In my own material slides of *S. stenotomum*, 37/131, and *S. Rybinii*, 43/8—36, have chiasma frequencies of about the same magnitude.

The chiasma frequencies within *S. Rybinii* show considerable variation. It is, however, impossible to say to what degree or extent this is dependent on environmental conditions. As mentioned earlier, the inbred ($I_1$) plants of this species segregated in morphological characteristics. There was also a wide variation in pollen fertility. Probably the variation in chiasma frequency is also at least partly of hereditary character. The slide of the plant, 43/8—35, had a remarkably high proportion of univalents, the mean number of univalents per cell being $1{,}00 \pm 0{,}2714$. The corresponding mean for the slide of the parental plant *S. Rybinii*, 39/8, is however $0{,}40 \pm 0{,}1835$. There is no significant difference between these means ($Df = 38$, $t = 1{,}831$), and the high univalent

frequency of this slide, as will be observed, may be due to accidental circumstances. This plant has also been classified as normal.

The low chiasma frequency of the highly fertile *S. verrucosum* is remarkable. As only one slide of one plant of this species has been analysed, general conclusions cannot be drawn.

Proof that certain diploid species differ from each other with respect to the chiasma frequency would be of some interest. In *Nicotiana* such differences are known, e. g. in *N. sylvestris* and *N. glutinosa* ring-bivalents are most common, while *N. longiflora* is characterized by rod-bivalents (GOODSPEED and AVERY, 1939; GOODSPEED, 1934). Within groups of more closely related *Nicotiana* species differences in chiasma frequencies are also known, e. g. between *N. alata* and *N. bonariensis* (AVERY, 1938). In these cases the different pairing behaviour has been explained by the structural chromosome differences between the species. Chiasma frequencies are determined by the position of the insertion region and the length of the arms of the chromosomes concerned (AVERY, 1938). In a number of plants reviewed by DARLINGTON (1937, pp. 399—404) differences in chiasma frequency and chromosome pairing are known which must be due to the segregation of specific genetic factors and not to particular structural differences. In dealing with the pairing behaviour of different *Solanum* species the possible existence of such gene-control must also be taken into account. At first, however, the question as to whether the diploid *Solanum* species of the section *Tuberarium* are or are not characterized by structural chromosome differences must be solved (cf. p. 93).

The crossing of *S. Rybinii* with *S. verrucosum* has been performed by EMME (1936 b) for the purpose of genetical studies, and by the present writer for cytological investigations. In the two slides of the $F_1$ hybrids, $43/8 \times 9_7$ and $43/8 \times 9_{13}$, the chiasma frequencies, as mentioned before, did not differ significantly from each other or from those found in the slides of either parent. The pairing behaviour of this hybrid seems to be normal (see Table 3), and does not indicate the existence of any considerable structural differences between the parental species. Thus the structural chromosome studies and meiotical investigations agree. The only indication observed by the author of a possible slight structural heterozygosity is the low terminalization coefficient of the hybrids. This was most marked in the rod-bivalents, all of which had one chiasma. Here the average coefficient was 0,88 for the parents and 0,71 for the hybrids.

The occurrence of structural heterozygosity in a diploid hybrid (multivalents, etc.) reveals the existence of structural differences between the idiograms of the parents. If, however, in a hybrid such aberrations from the normal bivalent pairing be missing, this does not definitely prove that the idiograms of the parents are really identical.

In the crosses *Lycopersicum esculentum* $\times$ *L. pimpinellifolium*

(HUMPHREY, 1937) and *Nicotiana longiflora* × *N. plumbaginifolia* (AVERY, 1938) the parental species differ in respect of the nucleolus-organizing chromosomes. Moreover, the mother species of these crosses have larger chromosomes. The chromosome pairing of the hybrids is fairly regular. In the $F_1$ hybrid of *Tragopogon pratensis* × *T. porrifolius* there is also a regular bivalent pairing, although there are considerable differences in the idiograms of the parental species (WINGE, 1938). Regularity of chromosome conjugation with large differences in the structure of the conjugating chromosomes characterizes the two hybrids *Nicotiana longiflora* × *N. alata* and *Nicotiana longiflora* × *N. Langsdorffii* (AVERY, 1938). In these hybrids between 10- and 9-paired species (2n = 20 and 18) mainly bivalents and univalents occur. There are also trivalents indicating the structural heterozygosity, but especially in the latter cross their frequency is low, and they might easily have been overlooked if a sufficient number of PMC's had not been examined. The pairing behaviour of these hybrids may partly be attributed to the peculiar chromosome morphology of *Nicotiana longiflora* (AVERY, 1938, pp. 186—187).

### e. Negative correlation of chiasma frequencies.

In the case of the slides of Table 3 investigations have also been made as to whether the variability in number of chiasmata found per bivalent is greater or less than the variability in the number of chiasmata formed per nucleus. The results of this investigation are given in Table 4. In five of the slides the inter- and intra-

TABLE 4. *Intra- and inter-cell variance of the individual plants of Table 3.*

| Plant | Sum of squares | | Mean square | |
|---|---|---|---|---|
| | Intra-cell (Df = 220) | Inter-cell (Df = 19) | Intra-cell (Df = 220) | Inter-cell (Df = 19) |
| *S. Rybinii*, 39/8 ..... | 60,3333 | 5,2667 | 0,2742 | 0,2772 |
| » 43/8—28 ..... | 51,5000 | 8,0833 | 0,2341 | 0,4254* |
| » » —34 ..... | 56,4167 | 4,0792 | 0,2564 | 0,2147 |
| » » —35 ..... | 66,8333 | 8,3500 | 0,3038 | 0,4395 |
| » » —36 ..... | 59,2500 | 4,3500 | 0,2693 | 0,2290 |
| *S. Ryb.* × *S. verr.*, $43/8 \times 9_7$ | 49,7500 | 7,8125 | 0,2261 | 0,4112* |
| » » $\times 9_{13}$ | 51,7500 | 6,5458 | 0,2352 | 0,3445 |
| *S. verrucosum*, 41/9 ..... | 44,1667 | 1,8167 | 0,2008* | 0,0956 |
| *S. stenotomum*, 37/131 ... | 52,6667 | 1,6292 | 0,2394** | 0,0858 |
| Total | 492,6667 | 47,9333 | 2,2393 | 2,5229 |
| Mean | (Df = 1980) | (Df = 171) | 0,2488 | 0,2803 |

nuclear mean square show no significant correlation of chiasma frequency, in two slides there is positive and in two slides negative correlation.

The negative correlation of the slides of *S. verrucosum*, 41/9, and *S. stenotomum*, 37/131, may indicate that the chromosome pairing of these plants is less affected by external conditions than is the pairing of the other plants. That external conditions may have different effects on pollen fertility has been shown on grafted plants of *S. tuberosum* and *S. acaule* (cf. p. 39). It is, however, somewhat astonishing that among the plants of *S. Rybinii* a slide of field No. 43/8—28 shows a significant positive correlation. This plant is highly pollen fertile and has a comparatively high chiasma frequency.

Negative correlation is explained by MATHER (1936 a, 1939) as due to a competition between bivalents during chiasma formation. The hypothesis of competition implies that there is an upper limit to the number of chiasmata which can form in any nucleus. In the case of the tomato the observations of UPCOTT (1935), according to MATHER (1939, p. 128), imply the absence of an upper limit. If this is also the case in the *Solanum* species investigated by the present writer, no correlation or positive correlation should be expected. On the other hand, if negative correlation should prove to be typical of ordinary diploid plants, the absence of correlation or a positive correlation could be expected in inbred plants and hybrids in analogy to the different behaviour of the population and inbred rye plants (MATHER and LAMM, 1935; LAMM, 1936). In my *Solanum* material the slides investigated of various categories are too few and the results too divergent to permit conclusions in the one or the other direction. Investigations on *Tulipa* (UPCOTT, 1939) and asynaptic rye (PRAKKEN, 1943) are also difficult to interpret on the basis of the hypothesis of competition, which after all might be wrong (cf. LAMM, 1944 a, p. 142).

### f. Meiosis in »aberrant plants».

#### *Simple cases.*

The meiotic behaviour of the five aberrant plants enumerated on page 15 has been more or less easy to interpret. In two of the plants the aberrations were complicated. Firstly, however, the simpler cases will be described.

In the PMC's of *S. stenotomum*, 37/114, a pair of univalents were frequently observed during I—M (Fig. 16). At I—A these univalents had a tendency to split (Fig. 17), and sometimes the division was completed (Fig. 18). MÜNTZING (1933 a) observed no absolutely certain case of division of univalents at the first division of triploid *Solanum*. At interphase one of these univalents often remained outside the daughter-nuclei.

Unclean I—A separation, characterized by a single bridge and a free fragment, was observed in some PMC's (Fig. 19). This well-known configuration indicates that the plant was heterozygous for an inversion.

Bridges were not observed at second division, which might have been expected in view of the comparatively low chiasma frequency of the genus *Solanum* and the not very frequent occurrence of I—A bridges in this plant (cf. RIBBANDS, 1937, p. 16 and UPCOTT, 1937, pp. 382—383).

Evidence of segmental interchange between non-homologous chromosomes was obtained in two plants of which meiosis in the PMC's was studied.

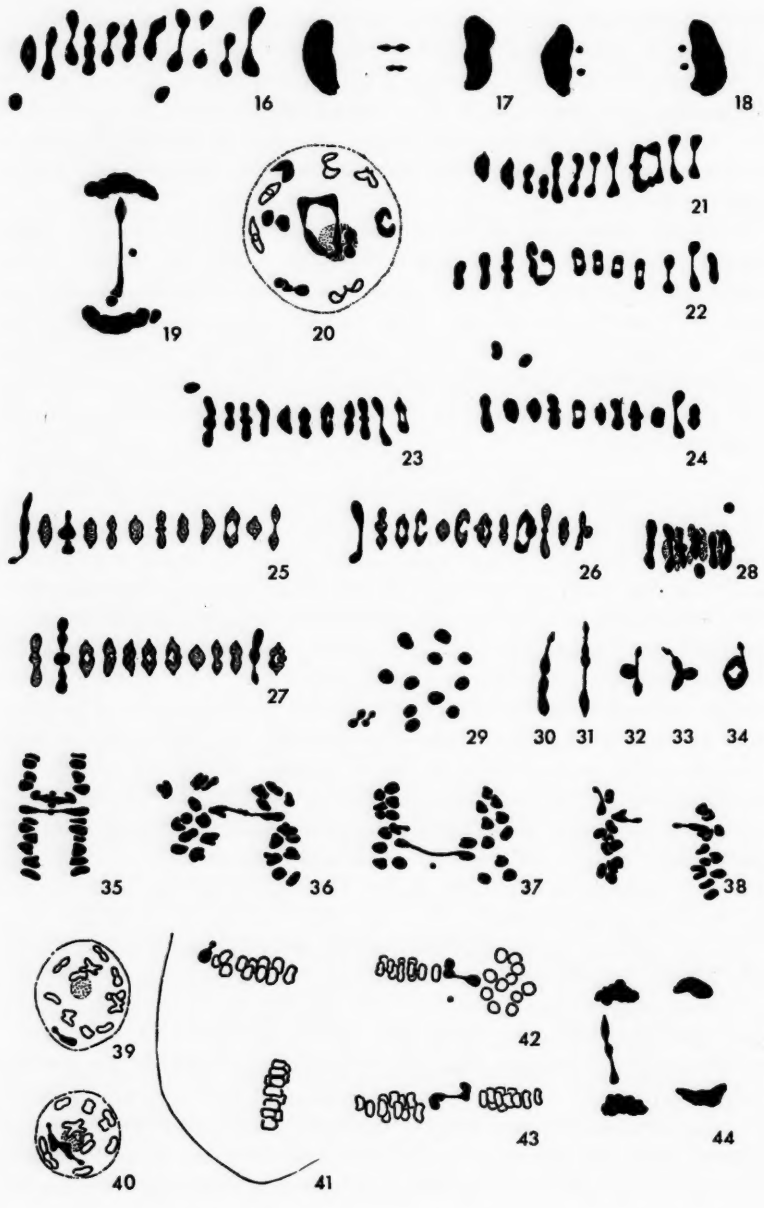
In *S. verrucosum*, 43/9—10, and *S. Rybinii*, 43/8—32, an amphibivalent was frequently observed at diakinesis and I—M. Fig. 20 shows a nucleus of the former plant at diakinesis, while Figs. 21—24 illustrate I—M of the latter plant. The amphibivalent can be seen in Figs. 20—22. As might be expected, cells were found where, instead of this configuration of four chromosomes, a trivalent plus a univalent (Fig. 23), two bivalents, or a bivalent plus two univalents (Fig. 24) occurred.

*Complicated cases.*

In *S. stenotomum*, 37/133, two pairs of unequal bivalents were observed at I—M. Figs. 25—27 show three complete PMC nuclei at I—M (the unequal bivalents are drawn in black). In a few cells the corresponding chromosomes occurred as pairs of univalents of unequal size (Figs. 28 and 29). These bivalents were frequently heteromorphic for a terminal satellite-like body. One of the partners within each of these bivalents obviously corresponds to the T-chromosomes found in inbred rye (see PRAKKEN and MÜNTZING, 1942). Figs. 30—34 show some types of these unequal bivalents, which are heterozygous for the T-tendency. In contradistinction to rye T-rings also occurred among the heteromorphic T-bivalents (Fig. 34). In one PMC two such T-rings were observed. Detachment of spherical chromatin bodies at the ends of the T-chromosomes, as was the case in rye, was not observed.

The unequal bivalents often had an interstitial chiasma (Figs. 25, 26, 27, 32 and 33), this probably being a consequence of arrested terminalization caused by the dissimilarity of the distal segments (cf. DARLINGTON, 1931 b, Fig. 16 A, and 1937, p. 511).

Difficulties of separation occurred at I—A (Figs. 35—38). The bridges might here be due to arrested terminalization, inversion (Fig. 37), and possibly also to other causes. The free fragments of the inversion-bridges were always rather small, and the inversion must have been terminal. Frequently the bridges were broken (Fig. 38). In Fig. 38,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besides a broken bridge, a T-chromosome can be seen in the left I—A group.

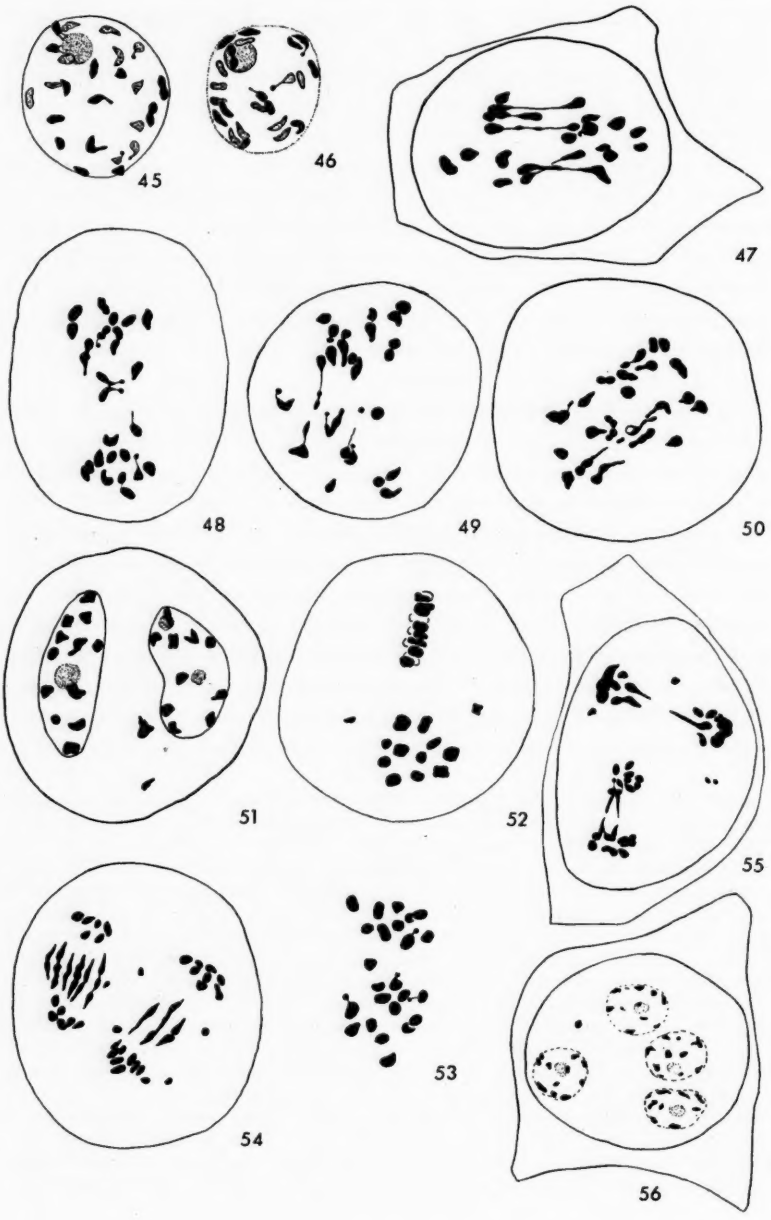
In some of the interphase nuclei the T-chromosomes could be distinguished. Figs. 39 and 40 show interphase nuclei from two different PMC's.

The T-chromosomes were sometimes also observed at II—M (Fig. 41). At this stage the two sister plates were frequently united by a persisting chromatin bridge (Figs. 42 and 43). The very small fragment chromatid was in most of these cases not observed. Rarely were bridges observed at II—A between the two chromosome groups of the same interphase nucleus (Fig. 44). The number of chromosomes in the two II—M plates was nearly always 12. Very few exceptions with a 13—11 distribution were found.

These unequal bivalents have probably originated through reciprocal translocation (cf. DARLINGTON, 1937, p. 162). Since the inequality was detectable at meiosis, the interchanged segments must have been of considerable size, and yet there were no amphibivalents. This indicates that the structural change must have been still more complicated, which is also evident from the occurrence of an inversion. For the explanation of the T-phenomenon reference may be made to PRAKKEN and MÜNTZING (1942). It may be of some interest to recall that the same phenomenon has also been found in inbred *S. Rybinii*.

As mentioned before, in the spring of 1941 seed obtained from selfing *S. Rybinii*, 39/8, was treated with colchicine. A chimaera was obtained from which purely diploid (41/8—1 a) and tetraploid (41/8—1 b)

Figs. 16—44. Meiosis in the PMC's of diploid *Solanum* plants having chromosomal aberrations. — Figs. 16—19, inversion in *S. stenotomum*. Fig. 16, I—M group (separately drawn). Fig. 17, lagging univalents. Fig. 18, divided univalents. Fig. 19, dicentric chromatid and fragment at I—T; Fig. 20, segmental interchange in *S. verrucosum*, a nucleus at diakinesis containing an amphibivalent; Figs. 21—24, segmental interchange in *S. Rybinii*, I—M groups (separately drawn) with an amphibivalent (Figs. 21 and 22), or instead of this a chain of three + a univalent (Fig. 23), a bivalent + two univalents (Fig. 24); Figs. 25—44, complicated aberrations in a plant of *S. stenotomum*. Figs. 25—27, I—M groups (separately drawn) each containing two pairs of unequal bivalents (drawn in black); Figs. 28—29, I—M groups each with two unequal univalents corresponding to one of the pairs of unequal bivalents; Figs. 30—34, some unequal bivalents from different cells. These bivalents are heteromorphic for a T-chromosome. Heteromorphic T-rod bivalents can also be seen in Figs. 25 and 27; Figs. 35—38, difficulties of separation at I—A, in Fig. 37 an inversion bridge with fragment, in Fig. 38 a broken bridge and a T-chromosome in the left I—A group; Figs. 39—41, T-chromosomes (drawn in black) during interphase and II—M; Figs. 42—43, II—M plates united by a persisting chromatin bridge; Fig. 44, bridge at II—A joining groups resulting from the same interphase nucleus. — × 2550.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tubers were selected. Meiosis of the PMC's was investigated in 1941 and 1942, and both years showed the same peculiar behaviour. Although one of the clones was diploid and the other tetraploid, they will be described together below.

Diakinesis and I—M were characterized by strong asynapsis. Fig. 45 shows a typical diakinesis nucleus of the diploid with complete asynapsis, whereas the pairing in the nucleus of Fig. 46 is exceptionally strong. In the nucleus last mentioned there are at least four bivalents. Some of the apparent pairs of univalents in this nucleus are perhaps in reality bivalents, as at this stage it is sometimes difficult to say whether a gap between two chromosomes is significant or not. The connecting chromatids may be rather thin and escape microscopical observation.

Figs. 57 and 58 are examples of diakinesis in the tetraploid. In Fig. 57, besides univalents, five bivalents and one quadrivalent can be seen, in Fig. 58 there are only univalents.

When compared with diakinesis of normal plants (Figs. 5 and 6), the chromosomes of the asynaptic plants at this stage seem to be less contracted. They sometimes remind one of chromosomes at mitotic metaphase. This behaviour is illustrated more especially by Fig. 58. In the short arms of some of the chromosomes terminal satellite-like bodies were observed not only at diakinesis but also during later stages. This is probably an expression of the same T-phenomenon as was described for *S. stenotomum*, 37/133, but in the present case there was an increase in the number of chromosomes. In one line of inbred rye a weak T-phenomenon was shown by a variable number of chromosome pairs (PRAKKEN and MÜNTZING, 1942). In the diploid *S. Rybinii*, 43/8—1 a, a maximum number of eight such chromosomes were counted.

At pro-metaphase the chromosomes were rather agglomerated. During the following stages of the first division most of the chromosomes appeared as univalents (Figs. 47—50 and 59—61), not arranged in a metaphase plate but spread over a, frequently, bent spindle (Fig. 61).

Figs. 45—56. Meiosis in the PMC's of a diploid asynaptic *S. Rybinii* plant. — Fig. 45, nucleus at diakinesis with only univalents; Fig. 46, nucleus at diakinesis with a few bivalents; Figs. 47—50, I-M—I-A stages. Besides univalents some apparent bivalents can be seen, but some of them may be sticking univalents. Figs. 47—50 also show bridges, broken bridges, fragments and T-chromosomes. Figs. 48—49 show the most common orientation of the T-ends. Fig. 48, a T-univalent at the equator with a tendency to divide; Fig. 51, interphase; Figs. 52—53, II—M, in Fig. 53 the chromosomes show the T-tendency; Figs. 54—55, II—A characterized by the occurrence of chromatin bridges, lagging chromosomes and fragments; Fig. 56, a tetrad. — × 2550.

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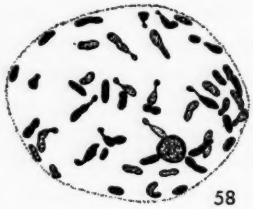

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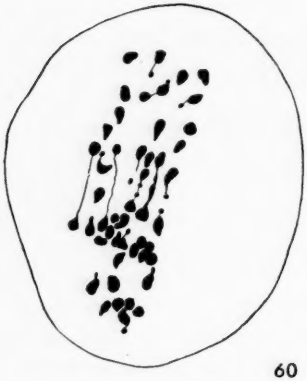

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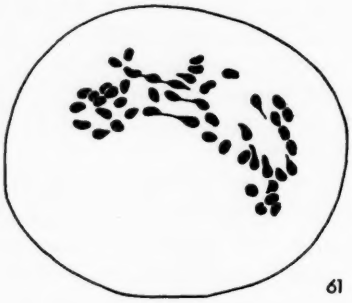

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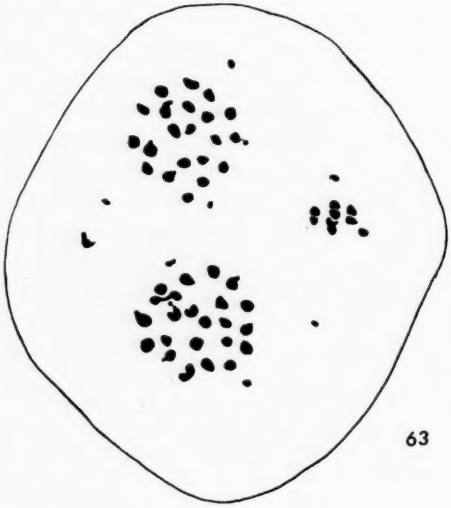

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just as in asynaptic *Datura* (BERGNER, CARTLEDGE and BLAKESLEE, 1934) and *Nicotiana* (BRIEGER, 1934). A few apparent bivalents also occurred, which can be seen from the figures. As will be discussed later, it is, however, possible that some of these apparent bivalents were sticking univalents. In the tetraploid, besides univalents and bivalents, trivalents and quadrivalents also occurred, although rarely (Figs. 61 and 62). The boundary between the I—M and I—A of these asynaptic plants is difficult to distinguish. During the first division, bridges (Figs. 47 and 59—61), broken bridges (Figs. 48—50 and 59—61), and fragments (Figs. 49, 50, 59 and 60), probably originating from these breakages, frequently occurred. As during diakinesis, the morphology of the univalents showed some variation (cf. Figs. 47 and 50). The univalents did not actually divide during the first division, though they showed an exceptional tendency towards division (see Fig. 48, the univalent at equator with terminal bodies).

As a rule the terminal satellite-like bodies were orientated towards the equator during the first division. In the diploid, out of 64 counted univalents with such bodies, 61 were orientated towards the equator. In this respect they behaved contradictorily to the T-arms in rye (PRAKKEN and MÜNTZING, 1942).

During interphase, univalents and fragments were frequently seen in the plasm outside the interphase nuclei (Fig. 51). Sometimes more than two interphase nuclei were formed as the result of irregular I—A separation.

At II—M the chromosomes were arranged in regular plates. Figs. 52 and 53 show II—M plates of the diploid, Fig. 63 of the tetraploid. The satellite-like bodies could sometimes be distinguished at this stage. Sometimes additional metaphase groups were seen (Fig. 63) originating from the additional interphase nuclei. A most remarkable observation was that the degree of secondary association at II—M among these asynaptic plants was rather low. This phenomenon will be discussed in the Chapter on secondary association.

Figs. 57—64. Meiosis in the PMC's of an artificial, autotetraploid asynaptic *S. Rybinii* plant (this plant and the plant corresponding to Figs. 45—56 were chimaeras). — Fig. 57, nucleus at diakinesis, besides univalents, showing one quadrivalent and some bivalents; Fig. 58, nucleus at diakinesis with only univalents, which are less contracted than in normal plants; Figs. 59—61, I-M—I-A stages. Fig. 60, some chromosomes showing T-tendency. Fig. 61 shows a bent spindle; Fig. 62, some trivalents and quadrivalents from different cells; Fig. 63, II—M groups showing an additional metaphase group, eliminated chromosomes and fragments, T-tendency of some of the chromosomes and a remarkably low degree of secondary association; Fig. 64, II—A with chromatin bridges. — × 2550.

At II—A chromatin bridges were frequently formed (Figs. 54 and 64), which as a rule were broken at the end of this stage (Fig. 55). Lagging chromosomes and fragments also frequently occurred (Figs. 54, 55 and 64). At the tetrad stage (Fig. 56) in the diploid most PMC's contained four nuclei, but additional micro-nuclei also occurred. These asynaptic plants were completely male-sterile. The anthers did not dehisce and they did not contain functional pollen.

Fig. 65. Leaves of diploid *S. Rybinii*. In the upper row of a normal plant, 39/8, in the lower row of an asynaptic plant, 41/8—1 a.

As mothers in crosses with normal plants these asynaptic plants also proved to be totally sterile. Embryological investigations were undertaken in the diploid. No functional embryo-sacs were formed. As a rule the EMC's degenerated after the first division.

The somatic divisions in root-tips were not aberrant. It should also be mentioned that in some morphological characteristics these asynaptic plants deviated considerably from their parents and sister-plants. Fig. 65 shows in the upper row leaves from the parent plant *S. Rybinii*, 39/8, and in the lower row leaves of the diploid asynaptic plant,

41/8—1 a. The leaflets of the latter plant are broadly oval, less acute at the apex, and have few intermittent smaller segments. In the tetraploid these characteristics were still more pronounced. Here the leaflets were also thicker and more rounded at the base. The anthers of diploid asynaptic plants were much shorter and thicker than in normal diploid plants of this species.

In several respects this asynaptic *S. Rybinii* is reminiscent of »sticky» maize (BEADLE, 1932). This type was also characterized by extensive bridge formation and fragmentation during both divisions of meiosis. In maize the stickiness of the chromosomes was due to a gene, which among other things also increased the frequency of gene mutation. From this point of view the appearance of aberrant leaf- and anther-shape in asynaptic *S. Rybinii* is of interest. This could either be due to mutation or to segregation, but I have never noticed the segregation of such types in progenies of diploid *Solanum* plants. Contrary to the case in *S. Rybinii* the sticky phenomenon in maize also appeared at somatic mitosis. Sticky mitosis has also been found in hybrids of *Chorthippus* by KLINGSTEDT (1939). Here, unstained gaps appeared in some chromosomes during meiosis. Perhaps these correspond to the thin connections between the short arms and their terminal bodies found in my asynaptic *Solanum*.

DARLINGTON and LA COUR (1940) suggest that the behaviour of sticky maize and *Chorthippus* might be due to a disturbed nucleic-acid metabolism of the chromosomes. By nucleic-acid starvation of chromosomes in *Trillium* similar conditions could be obtained. According to DARLINGTON and UPCOTT (1941), bridges without fragments at II—A are probably often due to sister reunion of true ends.

According to this hypothesis it might be assumed that in asynaptic *S. Rybinii* a gene mutation may be responsible for nucleic-acid starvation. At I—M the bridges without fragments could then be of two categories. One of these has already been described, namely bridge formation due to arrested terminalization in gemini. The other category is thought to be due to sticking univalents. At II—A sister chromatid reunion might be responsible for most of the bridges, this reunion in turn being probably caused by disturbed nucleic-acid metabolism.

It might be suspected that asynapsis and stickiness in these plants of *S. Rybinii* were a »dauer» modification caused by the colchicine treatment (cf. LEVAN, 1939, p. 129). However, other seed of *S. Rybinii* has been treated with colchicine without this effect on the plants raised. In rye PRAKKEN and MÜNTZING (1942) assume that the T-phenomenon

results from an interaction between the genotypical constitution of the various lines and a structural difference separating the lines with and without a T-chromosome pair. In the complicated cases of *S. Rybinii* such an explanation seems also to be acceptable as a working hypothesis.

C. NOTES ON SOME MORPHOLOGICAL ABNORMALITIES OF THE FLOWERS.

In the ordinary cultivated potato, *S. tuberosum*, morphological abnormalities on the flowers are common (ELLISON, 1936 a; v. KAUSCHE, 1937). It might be of some interest to note that such abnormalities are not confined only to *S. tuberosum* or to other polyploid species, e. g. *S. andigenum* and *S. demissum* (cf. v. KAUSCHE, 1937). In the cultivated diploid species investigated by the present writer such abnormalities also occurred in inbred progenies.

In *S. stenotomum*, 37/134, flowers with a calyx having six lobes and a corolla with six anthers frequently occurred.

In *S. Rybinii*, 43/8—30, the anthers of most of the flowers were pistilloid (l. c., pp. 120—123). Here the anthers developed from one to three green styles with typical stigmas.

Thus such abnormalities need not be an indication of hybrid origin (l. c., p. 138).

## 3. INVESTIGATIONS ON A TRIPLOID SPECIES.

### A. FERTILITY.

In the preceding Chapter the considerable variability of the pollen fertility between and within certain diploid *Solanum* species and hybrids has been treated. *A priori*, therefore, the same variability is to be expected in triploid species and hybrids.

Only one triploid species, *S. chaucha*, was studied by the present writer. The Svalöf material of this species had previously been investigated by MÜNTZING (1933 a). Although the Svalöf material probably consisted of one clone, single tubers were selected and propagated separately so that a number of undoubted clones were obtained. The two clones *S. chaucha*, 32/7—1 and 32/8—1, which thus are probably identical, were mainly used in my investigations.

Determinations of the percentage of good pollen in these clones during the summers of 1932—1934 have given the values submitted below.

| Clone | 1932 | 1933 | 1934 | Average pollen fertility |
|---|---|---|---|---|
| *S. chaucha*, 32/7—1 .... | 25 % | 11 % | 21 % | 19 % |
| » » , 32/8—1 .... | 17 » | 28 » | 18 » | 21 » |

The results of these determinations are in close agreement with those of MÜNTZING, who found an average pollen fertility of 25 per cent.

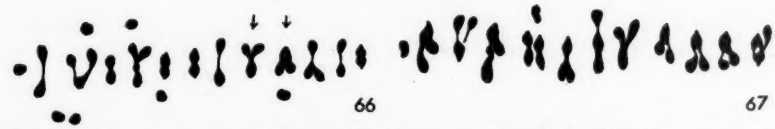
All attempts to self plants of these clones or to use them as male parents in crosses with diploid and tetraploid species were unsuccessful. As female partner *S. chaucha* was successfully crossed with *S. stenotomum* (2n = 24) and *S. tuberosum* (2n = 48). The hybrids will be described later.

The total male sterility of *S. chaucha* may have been due to unfavourable external conditions. Crosses performed by PROPACH with the triploid species *S. Commersonii* as male parent were exceptionally successful (v. OLÁH, 1938). In this species the pollen, according to VILMORIN (1929), contains 40—50 per cent good grains. Differences in pollen fertility between related triploid *Solanum* hybrids were observed by EMME (1936 a), but she did not determine the degree of fertility by counting samples of pollen. In autotriploid *Lycopersicum esculentum* JØRGENSEN (1928 a) counted 14 per cent good grains and LESLEY (1928) 13 per cent. Here the pollen did not function in spite of containing these percentages of apparently good grains.

SATINA and BLAKESLEE (1937 a) have shown that only a small part of the apparently good pollen of autotriploid *Datura* is capable of functioning, and here the triploid contained 50—60 per cent of this part. Of these apparently good grains only 15 per cent germinated on the stigma, and of this quantity two-thirds in their turn produced bursting pollen tubes.

## B. CYTOLOGY.

Apart from determining the somatic chromosome number on root-tip slides, the cytological investigations on *S. chaucha* have been restricted



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Figs. 66—67. I—M groups (separately drawn) in triploid *S. chaucha*. As to the trivalents of Fig. 66 indicated with arrows it is impossible to say whether they are of the chain or Y type. — × 2550.

to meiosis. Here my studies are only supplementary to those of MÜNTZING (1933 a).

Smears of the PMC's of *S. chaucha*, 32/8—1, were made in the summer of 1934. These were somewhat inferior to the slides made in

the following seasons. At that time I had not learned to dilute the fixation fluid sufficiently. Diakinesis was not fixed.

Figs. 66 and 67 show I—M in side view. Sometimes it was almost impossible to determine whether the trivalents were of the chain or the Y type (Fig. 66, the trivalents indicated with arrows). Consequently the chiasma frequency has not been determined. The configuration at I—M was examined in side view in 25 PMC's. The results are given in Table 5.

TABLE 5. *The types and frequency of chromosome pairing at I—M in a slide of S. chaucha, 32/8—1 ($2n = 36$).*

| No. of cells | Type of configuration | | | Remarks |
|---|---|---|---|---|
| | Univalents | Bivalents | Trivalents | |
| 1 | 10 | 10 | 2 | |
| 1 | 8 | 8 | 4 | |
| 2 | 7 | 7 | 5 | |
| 2 | 4 | 7 | 6 | autosyndetic |
| 1 | 6 | 6 | 6 | |
| 1 | 3 | 6 | 7 | » |
| 5 | 4 | 4 | 8 | |
| 2 | 6 | 3 | 8 | |
| 2 | 3 | 3 | 9 | |
| 5 | 2 | 2 | 10 | |
| 1 | 4 | 1 | 10 | |
| 2 | 1 | 1 | 11 | |
| Total 25 | 103 | 103 | 197 | |
| M | 4,1 | 4,1 | 7,9 | |
| ± m | 0,45 | 0,49 | 0,46 | |

The frequency of trivalents is remarkably high. On account of the death of the triploid plants I had no opportunity of conducting a re-investigation. In comparable triploids the following mean numbers of trivalents per cell have been found: *Lycopersicum esculentum* (UPCOTT, 1935) $4{,}9 \pm 0{,}16$; *S. acaule* × *S. chacoense* (PROPACH, 1937 b) $4{,}1 \pm 0{,}21$; *S. Commersonii* (v. OLÁH, 1938) $5{,}8 \pm 0{,}26$. (The last figures are somewhat doubtful, since v. OLÁH in 50 PMC's found 1838 instead of $50 \times 36 = 1800$ chromosomes, and from the total of 383 bivalents calculated a mean of 7,88 instead of $383 : 50 = 7{,}66$.) These means are all significantly lower than the mean $7{,}9 \pm 0{,}46$ of *S. chaucha*, 32/8—1. On the other hand, in certain autotriploid plants of other genera of the family Solanaceae the frequency of trivalents seems to be higher, e. g.

*Datura* (BELLING and BLAKESLEE, 1922) and *Nicotiana sylvestris* (GOODSPEED and AVERY, 1939).

The frequency of univalents in my slides of *S. chaucha* is remarkably low (cf. the papers cited above). On the other hand, MÜNTZING (1933 a), who analysed the frequency of univalents at I—M in his material of the same species, found a mean number of $2{,}5 \pm 0{,}21$ univalents per PMC, which is significantly lower than the mean $4{,}1 \pm 0{,}45$ found by the present writer ($t = 3{,}178^{**}$, $Df = 80$). This indicates either a high frequency of trivalents or a high degree of autosyndesis. These cells were not analysed with respect to the number of bivalents and trivalents. [According to LJUNGDAHL (1924), the term autosyndesis denotes pairing between chromosomes originating from the same gamete. Here, however, I use this term in the sense of pairing within the haploid ($n = 12$) chromosome set.]

If there is no autosyndesis, the sum of bivalents plus trivalents should not exceed 12, and the sum of trivalents plus univalents not be less than 12. In the triploid *Solanum* species or hybrids investigated by EMME (1936 a), PROPACH (1937 b), SCHWARZ (1937), v. OLÁH (1938), and IVANOVSKAJA (1941) autosyndetic pairing frequently occurs according to these authors. All the Russian workers especially have found a high degree of autosyndesis, which induces them to regard 6 and not 12 as the basic chromosome number of *Solanum*. They have, however, investigated polar views of I—M, which may easily lead to erroneous results. In triploid *Lycopersicum* autosyndetic pairing probably does not occur. The »mean configuration», which has been calculated on the basis of Table I in UPCOTT (1935), was $7{,}1_{I} + 7{,}0_{II} + 4{,}9_{III}$. (Tables I and III of her paper do not exactly agree with respect to the total number of trivalents.) It may also be mentioned here that LINDSTROM and KOOS (1931) found no pairing in haploid *Lycopersicum esculentum* between the twelve univalent chromosomes.

The author found a slight degree of autosyndesis (see Table 5) in *S. chaucha*, 32/8—1, in three PMC's. It is not excluded that these exceptions are due to faulty observations. The question as to whether or not autosyndesis occurs in triploids of the section *Tuberarium* is of some importance, and further investigations are therefore desirable.

At I—A divided univalents were very exceptionally observed. MÜNTZING (1933 a) has given a detailed description of the remaining facts concerning meiosis. Apart from the analysis of secondary association at II—M, which will be discussed later, I have made no additional ob-

servations of interest with respect to the meiotic behaviour of triploid *S. chaucha.*

## 4. INVESTIGATIONS ON NATURAL AND ARTIFICIAL TETRAPLOIDS AS WELL AS OCTOPLOIDS.

### A. SURVEY OF THE INVESTIGATIONS.

The tetraploids treated in this Chapter have been divided into two main groups, natural and artificial. The natural tetraploids are represented by *S. tuberosum*, *S. andigenum* and *S. acaule* var. *subexinterruptum*. The two former species belong to the group *Tuberosa*, the latter to the group *Acaulia*. The artificial autotetraploids were sister-plants to the diploid $I_1$ plants of *S. Rybinii* described earlier. Autotetraploids of *S. verrucosum* and of $F_1$ *S. Rybinii* × *S. verrucosum* were also obtained by colchicine treatment, but so far they have not been sufficiently investigated. Of *S. acaule* var. *subexinterruptum* artificial octoploids have been studied.

In this Chapter these tetraploids are compared with each other and with the commensurable diplo- and octo-ploids. The comparisons refer mainly to pollen fertility, cytological behaviour and certain morphological characteristics. *S. acaule* var. *subexinterruptum* will hereafter be referred to as *S. acaule* for the sake of brevity.

### B. FERTILITY.

#### a. Pollen fertility of grafted plants.

It has earlier been mentioned that certain crossings were performed on grafted mother-plants (cf. Fig. 68). Grafted as well as »normal» plants of *S. tuberosum*, Deodara, and of *S. acaule* were grown adjacently in open frames. During the summers of 1941—1943 some pollen samples of these plants were analysed. The pollen fertility and date of sampling are given in Table 6.

TABLE 6. *Percentage of good pollen in samples from »normal» and grafted Solanum plants.*

| Year / Date | | 1941 10/7 | 1942 2/7 | 1942 24/7 | 1942 30/7 | 1942 3/8 | 1943 20/6 | M % |
|---|---|---|---|---|---|---|---|---|
| *S. tub.* ..... | normal | 48 | 79 | 46 | 63 | 73 | 79 | 64,7 |
| Deodara .... | grafted | 43 | 73 | 33 | 53 | 56 | 51 | 51,5 |
| *S. acaule* var. .. | normal | | | 99 | 99 | 99 | 95 | 98,0 |
| *subex.* ..... | grafted | | | 99 | 98 | 99 | 97 | 98,3 |

As to Deodara the analysis of variance given under the figures of Table 6 shows that the pollen fertility of the grafted plants is significantly inferior to that of the normally grown plants. Further, there is a significant variation of the pollen fertility as between days.

| Source of variation | Df | Sum of squares | Mean square |
|---|---|---|---|
| Total ..... | 11 | 2528,92 | — |
| Days ..... | 5 | 1827,42 | 365,48* |
| Treatments ..... | 1 | 520,08 | 520,08* |
| Error ..... | 5 | 181,42 | 36,28 |

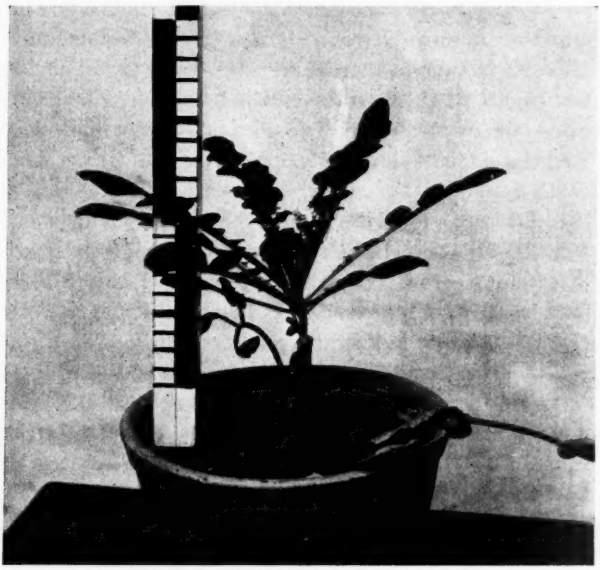


Fig. 68. *S. acaule* var. *subexinterruptum* cleft grafted on tomato.

The mean difference in pollen fertility between grafted and »normal» Deodara is $13{,}2 \pm 3{,}48$ per cent. *S. acaule*, on the contrary, seems to be very little affected by the environmental influences acting in this experiment. That the pollen fertility of *S. tuberosum* is rather sensitive to various environmental influences is known from several investigations (STOW, 1927; v. RATHLEF, 1932; KRANZ, BECKER and FINEMAN, 1939). The degeneration of the quality of the pollen in grafted Solanaceous plants is also reported by several authors (KOSTOFF, 1929, 1930 a; STELZNER and LEHMANN, 1939). Even if the grafting should

render certain difficult crossings possible, it usually does not increase the pollen fertility of the grafted plant.

The determinations of pollen fertility published in the present paper suffer from considerable uncertainty, as the effect of environmental conditions on the pollen fertility was usually not counterbalanced by taking two or more samples. Anyhow, it would have been rather difficult to carry out correct investigations, as the plants whose pollen fertility was to be compared often flowered at different times, e. g. diploids and corresponding artificial tetraploids.

### b. Fertility of different tetraploids.

Among the natural tetraploids the wild, autogamous species *S. acaule* is highly male and female fertile. In 1943 the pollen fertility of ten sister-plants of this species varied between 95 and 99 per cent, the mean percentage of good pollen being $96{,}4 \pm 0{,}48$. The seed content of three well developed berries of one of these plants was 234, 403 and 358 ($M = 332$).

In 1942 an octoploid ($2n = 96$) *S. acaule* plant was obtained (LAMM, 1943). The percentage of good pollen of this plant was 66. Fifteen sister-plants from the selfed progeny of this plant were investigated the following year. The pollen fertility varied between 55 and 86 per cent. The mean fertility was $73{,}1 \pm 2{,}49$ per cent. The difference in mean pollen fertility between these octoploids and the ten tetraploids was $23{,}3 \pm 2{,}53$ per cent ($t = 9{,}196^{***}$; $Df = 23$). The somatic chromosome numbers of only a few of these octoploids were controlled. In eighteen well developed berries, three harvested from each of six of these plants, the seed content per fruit varied between 33 and 106, the mean seed content being $74 \pm 4{,}97$. A comparison between tetraploid and octoploid *S. acaule* showed the decrease in fertility to be greater with respect to the seed production than with regard to the percentage of apparently good pollen. In the character last mentioned the decrease amounts to about 24 per cent.

Most varieties of *S. tuberosum* are rather male-sterile. v. RATHLEF (1932) has evaluated the pollen fertility of 183 varieties. Of these, 117 had less than 30 per cent good pollen. Such varieties were practically useless as male parents. The pollen fertility in *S. tuberosum* was rarely more than 60 per cent, according to v. RATHLEF. He found the pollen fertility of Deodara to be 41 per cent. In Table 6 the mean fertility of this variety is given as 64,7 per cent. The discrepancy is probably partly due to subjective differences in classifying the pollen as good or poor,

partly to environmental and other circumstances. V. KESSELER (1930) has shown that in Deodara only 12,4 per cent of the pollen could germinate on an artificial substrate. In autotetraploid *Datura* BUCHHOLZ and BLAKESLEE (1929) have shown that the apparently good pollen is only partly capable of function.

In 1943 the seed content of three well developed berries of Deodara was found to be 192, 163 and 185 (M = 180). For cytological investigations and for crossings a »number variety» of *S. tuberosum*, 36/209, was also used. This variety had a pollen fertility of 49 per cent. It produced plenty of berries. In certain triploid × tetraploid crosses the variety »Gammal röd» (Old red), 32/83, was used. The average pollen fertility of this variety was 60 per cent.

Artificial octoploids have also been induced in *S. tuberosum* (MÜNTZING and RUNQUIST, 1939; JOHNSTONE, 1939; STELZNER, 1941). These are usually more or less degenerated, especially with respect to their floral parts. STELZNER, however, obtained an octoploid of Pepo, and this was not absolutely female-sterile. The quality of the pollen was poor, but the degree of pollen sterility has not been published. *S. tuberosum* seems to suffer much more from the doubling of the chromosomes than *S. acaule*.

A rather vigorous and fertile clone of *S. andigenum*, 36/213, was chosen for crossings and cytological investigations. In eight samples of pollen from this clone the fertility varied between 85 and 96 per cent (M = 91). *S. andigenum* is generally more fertile than *S. tuberosum*, although varieties of the former species are also known that have a considerable degree of partial sterility (BUKASOV, 1933). On doubling the chromosome number of *S. andigenum* the degeneration was less than in *S. tuberosum* (JOHNSTONE, 1939).

From the diploid *S. Rybinii* seven artificial autotetraploid seed plants have been obtained. One of them, 41/8—1 b, which was completely sterile, has already been described. The pollen fertility of the six other plants is given below.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *S. Rybinii* | 43/65 | (2n = 48) | 43 | per | cent | good | pollen |
| » | » | » /67 | » | 16 | » | » | » | » |
| » | » | » /68 | » | 27 | » | » | » | » |
| » | » | » /69 | » | 0 | » | » | » | » |
| » | » | » /71 | » | 45 | » | » | » | » |
| » | » | » /72 | » | 21 | » | » | » | » |

The pollen fertility of their sixty diploid sister-plants is given in

Table 2. Of these diploid plants, thirty-three had more than 60 per cent good pollen.

The female fertility of these tetraploid *S. Rybinii* plants has not been properly determined. A great number of buds were taken for cytological investigation, and on each plant twenty-five flowers were emasculated and crossed with *S. tuberosum*, variety Deodara. Of 43/65 two fruits were obtained, containing 68 and 30 seeds, of 43/71 four fruits with a seed content of 22, 69, 56 and 110. The average seed content of these six fruits was 59. In the case of the other plants the crossings failed. A few crosses between the different tetraploid *S. Rybinii* plants also failed, but fruits from open pollinated flowers were harvested on *S. Rybinii* 43/65 and 43/71.

Autotetraploid plants of *S. Rybinii* have been investigated by RYBIN (1940) and WEICHSEL (1940), but the fertility of the tetraploids and the corresponding diploids has not been determined for the purpose of comparison.

Of *S. verrucosum* I obtained one autotetraploid plant, 43/54, the pollen fertility of which was 19 per cent. The fertility of the parent plant and corresponding diploid sister-plants is given in Table 2. The decrease in pollen fertility was here about 80 per cent, as compared with the mother-plant, which had 92 per cent good pollen. Lastly, two amphidiploids were obtained in the $F_1$ progeny of *S. Rybinii* $\times$ *S. verrucosum* (see Table 2). The pollen fertility of these two plants was remarkably high and amounted to 47 and 62 per cent.

The effect of doubling the chromosome number on pollen fertility has been studied in certain diploid species of the section *Tuberarium* by LIVERMORE and JOHNSTONE (1940). They found a decrease of about 30 per cent in *S. Jamesii* and *S. bulbocastanum*. In *S. chacoense* the decrease was about 90 per cent in comparison with the corresponding diploid. These species belong to the group *Stellata*, which is characterized by self-sterility (PROPACH, 1940).

In *Lycopersicum* the decrease of the pollen fertility of artificial autotetraploids as compared with corresponding diploids generally seems to amount to about 20 per cent (JØRGENSEN, 1928 a; LESLEY and LESLEY, 1930; LINDSTROM, 1932; LINDSTROM and HUMPHREY, 1933). The seed-setting was about 50—90 per cent less than that of the corresponding diploids. The most fertile tetraploid was the hybrid *L. esculentum* $\times$ *L. pimpinellifolium*, the most sterile was an autotetraploid *L. esculentum* originating from a haploid plant of this species (LINDSTROM and HUMPHREY, 1933).

The degree to which the fertility is reduced in connection with the doubling of the chromosomes may probably be more or less dependent on the morphology of the chromosomes, co-orientation of the multivalents and reaction against numerical chromosome unbalance of the gametes.

## B. CYTOLOGY.

### a. Somatic chromosomes.

*Investigations of chromosome morphology.*

Figs. 69—72 show somatic metaphase plates in *S. acaule* (2n = 48). The plate in Fig. 69 is from a root-tip of an untreated control plant, whereas the other three plates are from root-tips of plants which were cooled before fixation. These plants were subjected to the following treatments: 10° C, 69 hours, Fig. 70; 2° C, 144 hours, Fig. 71; no record of exact treatment, Fig. 72. The root-tips, with the exception of those corresponding to Fig. 72 which were fixed in medium FLEMMING, were fixed in modified NAVASHIN's solution. The result of the former fixation is the superior.

Fig. 73 shows a somatic metaphase plate of artificial octoploid *S. acaule* (2n = 96). The fixation is just good enough for counting the number of chromosomes.

Fig. 74 shows a somatic metaphase plate of artificial autotetraploid *S. Rybinii*, 43/71. The plant was slightly cooled before fixation (10° C, 48 hours).

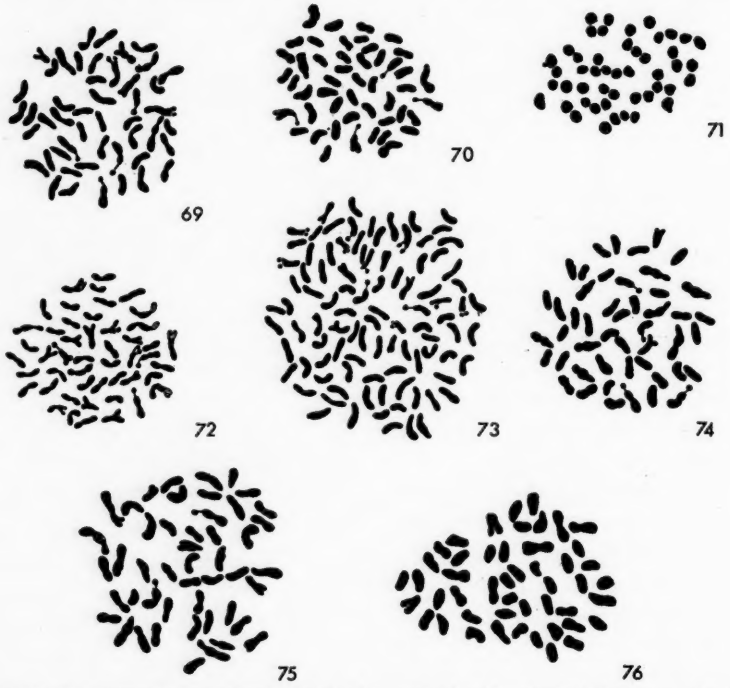
Figs. 75 and 76 are metaphase plates of the *S. tuberosum* variety Deodara. The former plate corresponds to an untreated control plant, the latter to the treatment at 2° C during 144 hours.

In *S. tuberosum* MEURMAN and RANCKEN (1932), ELLISON (1935) and ŠEPELEVA (1937) found two SAT-chromosomes. My observations agree with this statement (cf. Fig. 75). The number of SAT-chromosomes in autotetraploid *S. Rybinii* is four (Fig. 74), as could be expected from the observation of two SAT-chromosomes in the diploids. In *S. acaule* (2n = 48) the number of SAT-chromosomes is four (Figs. 69 and 70). As in the diploid species, besides the SAT-chromosomes, chromosomes with a secondary constriction in the long arm can be distinguished.

*The effect of cold treatment on the somatic chromosomes.*

In the slides of chilled root-tips the chromosomes were more or less contracted. There was usually variation in the degree of contraction between the cells of the same root-tip. Figs. 71 and 76 show the result of severe cold treatment (2° C, 144 hours) in *S. acaule* and *S. tuberosum* respectively. The effect of this treatment is super-contraction of the chromosomes, which has also been shown in other plants (cf. DARLING-

TON and LA COUR, 1940, p. 187). In *S. acaule* the chromosomes are contracted to a more or less spherical shape. This is not the case in *S. tuberosum*. As can be seen from Figs. 69—76, *S. acaule* has smaller chromosomes than *S. tuberosum* and *S. Rybinii*. It is remarkable that the contracted chromosomes of *S. acaule* (Fig. 71) show secondary association. In the chilled chromosomes of Fig. 72 the distal ends of




Figs. 69—76. Somatic metaphase plates of tetraploid and octoploid *Solanum* plants. — Figs. 69—72, *S. acaule*, unchilled chromosomes (Fig. 69), slightly chilled chromosomes (Fig. 70), strongly chilled chromosomes (Fig. 71), exact cold treatment not recorded (Fig. 72). Figs. 69—70 show four SAT-chromosomes. Fig. 71, secondary association of chilled supercontracted chromosomes. Fig. 72, probably heterochromatic segments in chilled chromosomes; Fig. 73, artificial octoploid *S. acaule*; Fig. 74, artificial autotetraploid *S. Rybinii*, slightly chilled, four SAT-chromosomes; Figs. 75—76, *S. tuberosum*, Deodara, unchilled metaphase with two SAT-chromosomes (Fig. 75), strongly chilled supercontracted chromosomes (Fig. 76). — × 2550.

the long arms of four secondarily constricted chromosomes were weakly stained. These ends are probably heterochromatic (cf. DARLINGTON and LA COUR, 1940, 1941). It is surprising that although the effect of

cold treatment is generally a contraction of the chromosomes, the effect occasionally seems to be dissimilar chromosomal activity (Fig. 72). This last observation, however, should be controlled by more extensive experiments.

### b. General meiotic behaviour.

*S. acaule var. subexinterruptum.*

In *S. acaule* (2n = 48) meiosis is characterized by a regular pairing into bivalents (PROPACH, 1937 b; LAMM, 1943). Figs. 77 and 78 show two nuclei at diakinesis. As a rule, two pairs of chromosomes are attached to the nucleolus (Fig. 77), but in Fig. 78 there is only one pair.

Most of the bivalents are joined by two chiasmata, but bivalents with one chiasma also occur (Fig. 78).

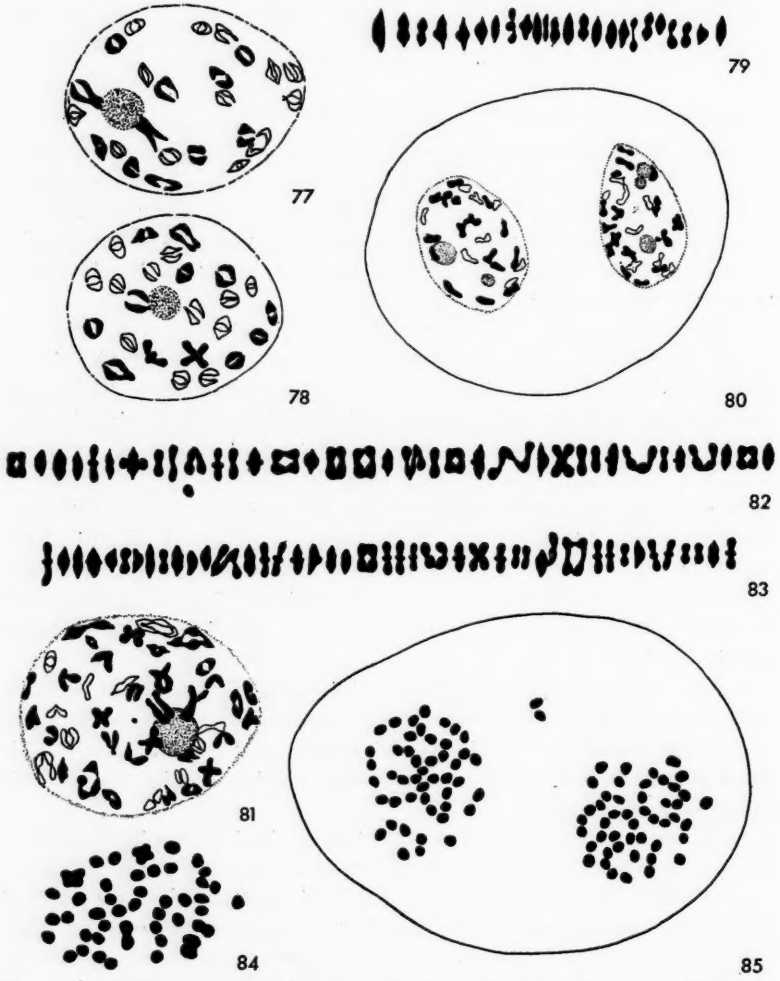
The very regular appearance of I—M is illustrated by Fig. 79, which also shows that the terminalization is not complete in all bivalents. Analysis of I—M in side view is somewhat difficult because of the high chromosome number, and it is sometimes difficult to distinguish between ring- and rod-bivalents. Because of this and for the same reasons as were mentioned in connection with the triploids the chiasma frequencies of the tetraploids have not been determined. Twenty PMC's at I—M contained bivalents with the exception of one pair of univalents (cf. Table 8). No doubt ring-bivalents are the more common. The rod-bivalents observed frequently had a subterminal chiasma. Subterminal chiasmata also occurred in the ring-bivalents. Multivalents were not at all observed.

Fig. 80 shows a typical PMC at interphase. Very frequently the interphase nuclei contain two or three nucleoli. In one case the two interphase nuclei of a PMC contained six and four nucleoli. This particular slide was, however, not FEULGEN-stained, which renders this observation uncertain.

II—M is perfectly regular and characterized by a high degree of secondary association (cf. p. 73). At the sporad stage (as to this term cf. WEBBER, 1933) only tetrads occur and from one to four nucleoli have been counted in the single nuclei of a tetrad (cf. Table 7). In view of the number of SAT-chromosomes only two nucleoli should be expected.

In octoploid *S. acaule* (2n = 96) meiotical studies were still more difficult because of the high chromosome number. The chromosome pairing could be analysed in seven PMC's. In three of them the pairing

was studied in polar views of I—M. These observations in polar view are somewhat dubious, since secondarily associated bivalents may sometimes be mistaken for quadrivalents.




Figs. 77—85. Meiosis in the PMC's of *S. acaule*. — Figs. 77—80, normal tetraploid *S. acaule* characterized by bivalent pairing; Figs. 77—78, nuclei at diakinesis; Fig. 79, I—M group (separately drawn); Fig. 80, interphase; Figs. 81—85, artificial octoploid *S. acaule*; Fig. 81, nucleus at diakinesis; Figs. 82—83, I—M groups (separately drawn); Fig. 84, I—M in polar view; Fig. 85, II—M groups. — × 2550.

The results of these observations are given below:

| PMC No. | Configuration | Observed stage of meiosis |
|---|---|---|
| 1..... | $7_{IV} + 34_{II}$ | Diak. (Fig. 81) |
| 2..... | $7_{IV} + 1_{III} + 32_{II} + 1_{I}$ | » |
| 3..... | $12_{IV} + 1_{III} + 22_{II} + 1_{I}$ | I—M, side view (Fig. 82) |
| 4..... | $6_{IV} + 36_{II}$ | » » » ( » 83) |
| 5..... | $6_{IV} + 36_{II}$ | » polar » |
| 6..... | $5_{IV} + 38_{II}$ | » » » ( » 84) |
| 7..... | $12_{IV} + 24_{II}$ | » » » |
| Total | $55_{IV} + 2_{III} + 222_{II} + 2_{I}$ | |

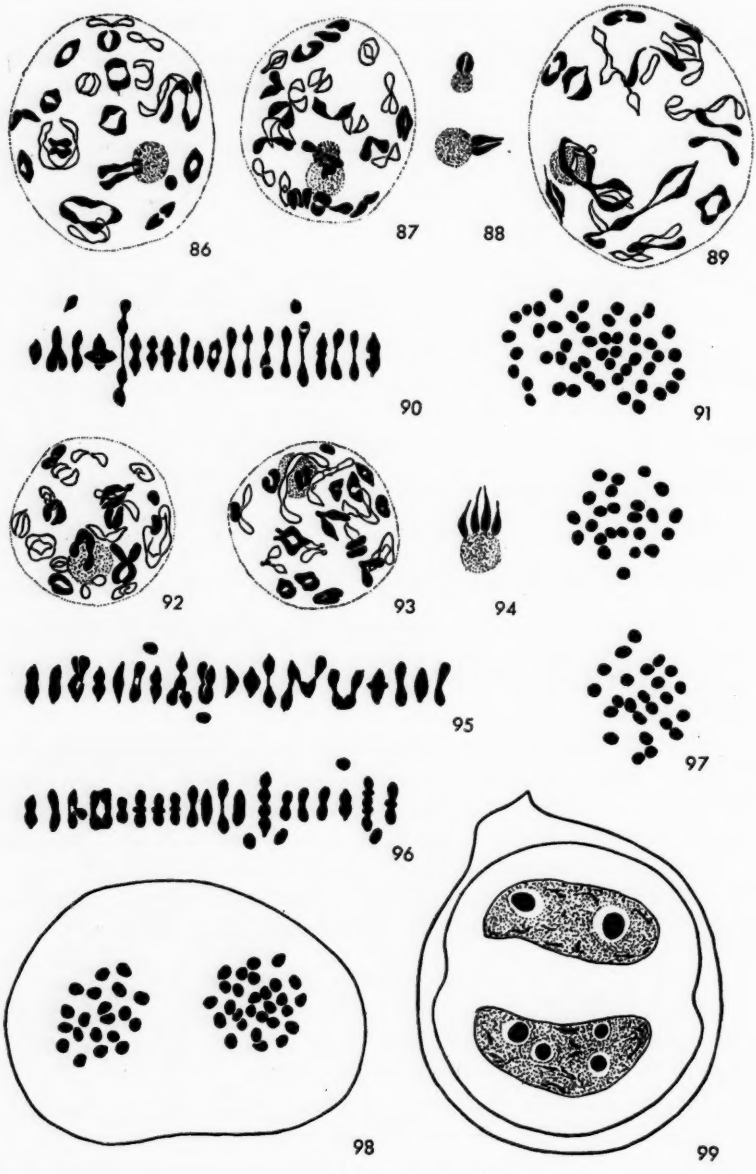
At diakinesis eight chromosomes can be seen attached to the nucleolus (Fig. 81). Occasionally at diakinesis nuclei with two nucleoli occur. Multivalents with more than four chromosomes were not observed. The percentage of observed quadrivalents calculated on the total number of potential quadrivalents is $35\left(\frac{55 \times 100}{7 \times 24} = 35\right)$. In autotetraploid *Lycopersicum esculentum* investigated by UPCOTT (1935) the corresponding percentage was 32 $\left(\frac{193 \times 100}{50 \times 12} = 32\right)$. It should, however, be pointed out that the frequency of quadrivalents found in octoploid *S. acaule* is not very representative, since the PMC's were not chosen at random and rather few were analysed, some of these being studied in polar view.

At II—M lagging chromosomes were frequently observed (Fig. 85). The distribution of chromosomes between the two II—M plates of the PMC's have not been investigated. From somatic chromosome counts on $F_1$ hybrids between octoploid *S. acaule* and ordinary tetraploid *S. tuberosum* it can be concluded that the chromosome distribution in the EMC's is somewhat irregular (cf. p. 90). The number of nucleoli in the tetrad nuclei has been determined and is given in Table 7.

### *S. tuberosum and S. andigenum.*

The investigations of meiosis in *S. tuberosum* are restricted to the two varieties Deodara and 36/209. With respect to fertility these are superior to most of the varieties of this species.

Fig. 86 shows a nucleus of 36/209 at diakinesis (configuration: $3_{IV} + 1_{III} + 16_{II} + 1_{I}$). Two chromosomes are associated with the nucleolus. HEYN (1930) sometimes observed two nucleoli at diakinesis in *S. tuberosum*. This observation has been confirmed by the present in-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vestigation. Fig. 87 (configuration: $2_{III} + 20_{II} + 2_{I}$) and Fig. 88 illustrate the occurrence of two nucleoli at diakinesis in Deodara. In the former case there is one univalent associated with each of the two nucleoli, in the latter each of the nucleoli is associated with a bivalent. As mentioned before, the somatic chromosome complement of *S. tuberosum* contains two SAT-chromosomes. Fig. 88 shows that other chromosomes than the SAT-chromosomes may occasionally form a nucleolus. Fig. 89 also exemplifies diakinesis in Deodara (configuration: $4_{IV} + 2_{III} + 12_{II} + 2_{I}$). It also indicates that not only chain- or ring-quadrivalents occur but also quadrivalents with multiple chiasmata, two of which can be seen in Fig. 89. The differences in size between the illustrated diakinesis nuclei may be due to a different orientation of the PMC's in the slides.

Fig. 90 shows a I—M in Deodara (configuration: $2_{IV} + 2_{III} + + 16_{II} + 2_{I}$). One of the quadrivalents is of the chain type and the other of a more rarely occurring type (cf. DARLINGTON, 1937, Fig. 40, Type 18), which, however, is also illustrated by Fig. 89.

FEULGEN-stained slides of Deodara have been studied at interphase and at the tetrad stage. At interphase there is usually one large nucleolus per nucleus, but sometimes there are two or three and very rarely four nucleoli. The frequency of nucleoli in the tetrad nuclei is given in Table 7.

In Deodara the II—M groups were frequently fused (Fig. 91), giving rise to dyad formation as earlier shown by HEYN (1930). This author found 23 per cent of dyads in Deodara at the sporad stage. In *S. tuberosum*, 36/209, II—M was rather regular.

In *S. andigenum*, 36/213, two nucleoli were sometimes observed in the nuclei at diakinesis. Meiosis of this plant was fairly regular.

### *S. Rybinii* ($2n = 48$).

Smears were taken from the anthers of all the artificial autotetraploids and the chromosome numbers were counted at II—M. The chromosome numbers of these plants were thus determined both as regards

Figs. 86—99. Meiosis in the PMC's of *S. tuberosum* and artificial autotetraploid *S. Rybinii*. — Figs. 86—91, from *S. tuberosum*; Figs. 86—87, nuclei at diakinesis; Fig. 88, two nucleoli each associated with one bivalent (separately drawn from the same diakinesis nucleus); Fig. 89, nucleus at diakinesis; Fig. 90, I—M group (separately drawn); Fig. 91, fusion of II—M groups in Deodara.
Figs. 92—98, *S. Rybinii*, 43/65; Figs. 92—93, nuclei at diakinesis; Fig. 94, separately drawn diakinesis nucleolus associated with four chromosomes; Figs. 95—96, I—M groups (separately drawn); Figs. 97, II—M plates from a PMC showing the chromosome distribution 24—24; Fig. 98, II—M plates showing the distribution 20—28; Fig. 99, dyad from *S. Rybinii*, 43/72. — × 2550.

root-tips and PMC's. Really good fixations of meiosis were, however, only obtained of *S. Rybinii*, 43/65 and 41/8—1 b. Meiosis of the latter plant, which was asynaptic, has been described earlier.

Diakinesis in *S. Rybinii*, 43/65, is illustrated by Figs. 92 and 93 (configurations: $4_{IV} + 15_{II} + 2_{I}$ and $2_{IV} + 2_{III} + 16_{II} + 2_{I}$). The multivalents of Fig. 93 are all of the chain type. In Fig. 92 there are two chain-quadrivalents, one ring-quadrivalent and one quadrivalent with a multiple chiasma (DARLINGTON, 1937, Fig. 40, Type 15). In the nucleus of Fig. 92 two bivalents are associated with the nucleolus. Fig. 94 shows four chromosomes, probably united into a quadrivalent, associated with the nucleolus at diakinesis.

Figs. 95 and 96 exemplify I—M's of the same plant (configurations: $5_{IV} + 13_{II} + 2_{I}$ and $2_{IV} + 2_{III} + 15_{II} + 4_{I}$). In Fig. 96 one trivalent and four rod-bivalents have subterminal chiasmata.

Figs. 97 and 98 illustrate the II—M of *S. Rybinii*, 43/65. In Fig. 97 the numerical distribution of chromosomes between the two II—M plates is balanced (24—24), in Fig. 98 rather unbalanced (20—28). The sporad stage of this plant was quite normal. Also here an analysis of the frequency of nucleoli was made (cf. Table 7).

One of the autotetraploid sister-plants, *S. Rybinii*, 43/72, at the second division, behaved in the same way as Deodara. Of 160 PMC's studied at the sporad stage no fewer than 25 (= 16 per cent) contained dyads (cf. Fig. 99). Here, too, the dyad formation was caused by the fusion of II—M spindles. It ought to be mentioned that other methods of dyad formation in *Solanum* are also known (cf. FUKUDA, 1927; STOW, 1927; HEYN, 1930; BLEIER, 1931 a; ELLISSON, 1936 a; LAMM, 1941).

With reference to the autotetraploids of *S. Rybinii* it is most interesting to see that certain types of abnormal meiosis occur, which are frequently met with in *S. tuberosum*. We have seen that *S. Rybinii*, 43/72, showed the same type of dyad formation as Deodara. *S. Rybinii*, 41/8—1 b, was asynaptic, a phenomenon frequently met with among varieties of *S. tuberosum* (FUKUDA, 1927; HEYN, 1930; LONGLEY and CLARK, 1930; ELLISON, 1936 a). Such meiotical aberrations in *S. tuberosum* are thus no proof of a hybrid origin of this species.

### c. Frequency of nucleoli in the tetrad nuclei.

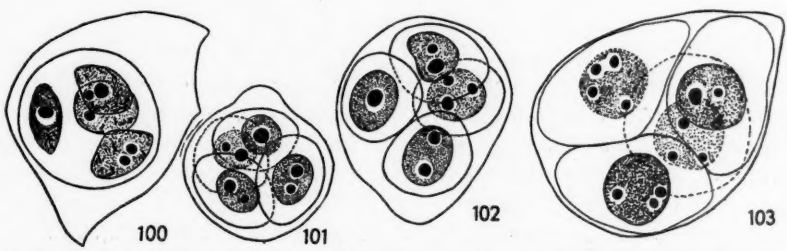
These frequencies were primarily investigated with a view to seeing whether the results of colchicine treatment could be tested in a simple and convenient way. For this investigation only sporads with four nuclei, i. e. true tetrads, were chosen. Table 7 shows that there is a

positive correlation between the number of SAT-chromosomes and the number of nucleoli in the tetrad nuclei.

TABLE 7. *The frequency of nuclei with various numbers of nucleoli in the tetrads of some Solanum plants.*

(Of each plant 100 tetrads were investigated.)

| Plant | Somatic chromosomes | No. of SAT-chromosomes | No. of nucleoli per nucleus 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|---|
| *S. Rybinii*, 39/8 ..... | 24 | 2 | 400 | | | |
| » *verrucosum*, 41/9 .... | » | » | 389 | 11 | | |
| » *tuberosum*, Deodara .. | 48 | » | 367 | 33 | | |
| » *andigenum*, 36/213 ... | » | » | 376 | 24 | | |
| » *Rybinii*, 43/65 ..... | » | 4 | 321 | 74 | 5 | |
| » » , 43/72 ..... | » | » | 285 | 107 | 8 | |
| » *verrucosum*, 41/9 ..... | » | » | 380 | 90 | 2 | |
| » *acaule*, 42/231 ..... | » | » | 286 | 110 | 4 | |
| » » , 42/232— 1 ... | 96 | 8 | 253 | 114 | 33 | |
| » » , 43/232—11 ... | » | » | 200 | 157 | 42 | 1 |




Figs. 100—103. Tetrads stained for counting of nucleoli. — Fig. 100, young tetrad of artificial autotetraploid *S. Rybinii*, 43/72; Fig. 101, tetrad of *S. tuberosum*, Deodara; Fig. 102, tetrad of tetraploid *S. acaule*; Fig. 103, tetrad of artificial octoploid *S. acaule*. — × 1275.

In the diploids the tetrad nuclei only rarely contain two nucleoli, which are then of different size. In the corresponding autotetraploids two nucleoli of about the same size frequently occur. Fig. 100 shows a young tetrad of *S. Rybinii*, 43/72, where two of the nuclei each contain one nucleolus, the two others two nucleoli. A dyad of the same plant is shown in Fig. 99.

In *S. tuberosum*, Deodara, and in *S. andigenum*, 36/213, two nucleoli were more common than in the diploids (cf. Table 7). In such nuclei the two nucleoli generally differ in size, as illustrated in Fig. 101, which shows a tetrad of Deodara. It is of interest to note that these

tetraploid plants with two SAT-chromosomes have a higher frequency of nuclei with two nucleoli than the diploid plants with the same number of SAT-chromosomes. Even where this difference is less pronounced, e. g. between *S. verrucosum*, 41/9, and *S. andigenum*, 36/213, the heterogeneity is significant ($\chi^2 = 5{,}049^*$; Df = 1). Thus it seems as if either (1) the chromosomes which in addition to the SAT-chromosomes probably have the capacity of nucleolus organization are more active in these tetraploid species or (2) there is less fusion of nucleoli in the tetraploid because of the greater size of the nucleus. Perhaps one pair of these additional nucleolus-organizing chromosomes were originally SAT-chromosomes which, although having lost their satellites in the course of phylesis, still correspond to the second pair of SAT-chromosomes in the experimental autotetraploids.

In ordinary tetraploid *S. acaule* the frequency distribution of nucleoli is about the same as in autotetraploid *S. Rybinii*. In nuclei with two nucleoli these may be of the same or different size, as is shown by Fig. 102. The tetrads of octoploid *S. acaule* are characterized by the comparatively frequent occurrence of nuclei with three nucleoli (cf. Fig. 103).

### d. Primary pairing of the tetraploids.

Table 8 gives a survey of chromosome pairing at diakinesis and I—M in the plants investigated. For comparison the corresponding values obtained by UPCOTT (1935) in autotetraploid *Lycopersicum esculentum* are also included.

The difference between *S. acaule* and the other tetraploids of Table 8 is quite obvious. *S. acaule* is characterized by bivalent pairing. This type of pairing, although characteristic of allopolyploids, may sometimes occur in autotetraploids if there is a low chiasma frequency (cf. UPCOTT, 1939). As mentioned earlier, ring-bivalents are rather common in *S. acaule*, and the chiasma frequency of this species is probably comparatively high. The pairing behaviour thus indicates allopolyploidy, which, however, will be discussed more exhaustively later.

With the exception of *S. acaule* the pairing in the tetraploids of Table 8 is characterized by the occurrence of multivalents. Since the nature of the tetraploidy in *S. tuberosum* has been the object of much speculation, it is of special interest to see whether there are significant differences between the representatives of this species and the artificial true autotetraploid *S. Rybinii*. In Table 9 the numbers of multivalents

TABLE 8. *Survey of chromosome pairing at diakinesis and I—M in the tetraploid plants investigated.*

| Tetraploids investigated | No. of PMC's | Stage of meiosis | Mean No. per PMC Uni-valents | Bi-valents | Tri-valents | Quadri-valents |
|---|---|---|---|---|---|---|
| **Natural tetraploids** | | | | | | |
| **Allopolyploid type of pairing** | | | | | | |
| *S. acaule* ..... | 20 | I—M | 0,10 | 23,95 | | |
| **Autopolyploid type of pairing** | | | | | | |
| *S. tuberosum*, Deodara .... | 10 | Diak. | 1,50 | 19,20 | 1,10 | 1,20 |
| » » , » .... | 20 | I—M | 1,60 | 17,30 | 1,20 | 2,05 |
| » » , 36/209 ..... | 10 | Diak. | 1,19 | 17,60 | 1,10 | 1,90 |
| » » , » ..... | 20 | I—M | 1,05 | 20,30 | 0,45 | 1,25 |
| » *andigenum*, 36/213 ..... | 20 | I—M | 1,25 | 19,55 | 0,75 | 1,35 |
| **Artificial tetraploids** | | | | | | |
| **Autopolyploid type of pairing** | | | | | | |
| *S. Rybinii*, 43/65 ..... | 10 | Diak. | 1,30 | 16,30 | 0,70 | 3,00 |
| » » , » ..... | 20 | I—M | 3,25 | 14,10 | 1,05 | 3,35 |
| **For comparison (UPCOTT, 1935)** | | | | | | |
| *Lycopersicum esculentum* ... | 50 | Diak. | 0,56 | 15,80 | 0,08 | 3,86 |

TABLE 9. *Number of multivalents (III + IV) per cell in S. tuberosum and autotetraploid S. Rybinii.*

| No. of multivalents per cell | *S. tuberosum* Deodara | | 36/209 | | *S. Rybinii* 43/65 | |
|---|---|---|---|---|---|---|
| | Diak. | I—M | Diak. | I—M | Diak. | I—M |
| 0 ..... | 1 | 1 | | 1 | | |
| 1 ..... | 2 | 1 | 1 | 8 | | |
| 2 ..... | 3 | 3 | 4 | 8 | 1 | 1 |
| 3 ..... | 3 | 5 | 1 | 2 | 2 | 4 |
| 4 ..... | | 7 | 3 | 1 | 6 | 6 |
| 5 ..... | | 3 | | | 1 | 5 |
| 6 ..... | 1 | | 1 | | | 3 |
| 7 ..... | | | | | | 1 |
| M | 2,30 | 3,25 | 3,00 | 1,70 | 3,70 | 4,40 |

per cell in *S. tuberosum*, Deodara, *S. tuberosum*, 36/209, and *S. Rybinii*, 43/65, are given.

The figures in Table 9 have been tested by the analysis of variance in accordance with the method described by SNEDECOR (1938, pp. 233—235) for proportional subclass numbers. The result of this analysis is given below.

*Analysis of variance referring to Table 9.*

| Source of variation | Df | Sum of squares | Mean square |
|---|---|---|---|
| Between stages (Diak.—I—M | 1 | 0,27 | 0,27 |
| » plants | 2 | 62,96 | 31,48 |
| *S. tuberosum—S. Rybinii* | 1 | 53,36 | 53,36*** |
| Within *S. tuberosum* (Deodara—36/209) | 1 | 9,60 | 9,60 |
| Interaction, stages × plants | 2 | 20,28 | 10,14** |
| Stages × (*S. tuberosum—S. Rybinii*) | 1 | 3,41 | 3,41 |
| » × (Deodara—36/209) | 1 | 16,87 | 16,87** |
| Within plants within stages (= error) | 84 | 130,95 | 1,56 |
| Total | 89 | 214,46 | |

The difference in the frequencies of multivalents between the means obtained for the two varieties of *S. tuberosum* and *S. Rybinii*, 43/65, is significant. In order to test this significance the variance ratio between the mean squares 53,36 and 1,56 has been calculated. The corresponding variance for interaction (3,41) is not significant. The inter-plant difference within *S. tuberosum*, however, is not significant, since the variance (9,60) is less than the significant variance for the corresponding interaction (16,87). These results, however, cannot be generalized until further investigations have been made on a greater number of representatives of these natural and experimental tetraploids. The similarity in pairing between *S. tuberosum* and *S. andigenum*, 36/213, can be seen from Table 8.

Certain results of meiotical investigations on *S. tuberosum* show the difficulties of estimating the tendency of multivalent formation. This tendency seems to be influenced to some extent by environment. STOW (1927) has pointed out the influence of temperature on chromosome pairing. According to HEYN (1930), BLEIER (1931 a), BAYLISS (1936) and ELLISON (1936 a), he has, however, overestimated this influence. Recently, the pairing in the *S. tuberosum* variety Flour-ball has been studied by CADMAN (1943). He found the mean number of multivalents per PMC to be not less than $5{,}2 \pm 0{,}212$. In some cells nine multivalents were observed. CADMAN has, however, given a rather brief report of his investigations, and it is difficult to judge of the correctness of his observations. In *S. Rybinii*, 43/65, the mean number of multivalents per cell at diakinesis and I—M is $4{,}2 \pm 0{,}215$. This estimate is based on 30 PMC's, whereas CADMAN analysed 70 PMC's. The difference between

these two means is significant ($t = 3{,}545^{***}$, $Df = 98$). When studying the I—M of meiosis in autotetraploid *S. Rybinii*, RYBIN (1940) only found bivalents, which seems rather extraordinary.

Table 8 also includes *Lycopersicum esculentum* (UPCOTT, 1935). In comparison with the *Solanum* plants *Lycopersicum esculentum* has a very low frequency of trivalents. Among *Solanum* a potential quadrivalent is more often expressed as a trivalent plus a univalent than in *Lycopersicum esculentum*. In the latter species the mean number of quadrivalents per cell was $3{,}9 \pm 0{,}251$ (UPCOTT, 1935). This does not differ significantly from the mean number of multivalents per cell in *S. Rybinii*, 43/65.

Neither in *S. tuberosum* nor in other tetraploid *Solanum* types investigated by the author did primary associations of higher order than quadrivalents occur. In *S. tuberosum*, however, numerous authors have found higher multivalents than these (STOW, 1927; MEURMAN and RANCKEN, 1932; ELLISON, 1936 a; CADMAN, 1943). MEURMAN and RANCKEN (l. c., p. 21) assume that these configurations are due to segmental interchange. This seems quite reasonable, since, as shown in this paper, segmental interchange occurs in the diploid species. It

TABLE 10. *Frequencies of different types of quadrivalents in Solanum and Lycopersicum.*

| Plants | No. of quadrivalents | | | |
|---|---|---|---|---|
| | Chains | Rings | With multiple Xta | Total |
| *Solanum* | | | | |
| Natural tetraploids | | | | |
| *S. tuberosum*, Deodara ..... | 27 | 10 | 16 | 53 |
| » » , 36/209 ..... | 21 | 18 | 5 | 44 |
| » *andigenum*, 36/213 ..... | 13 | 9 | 5 | 27 |
| Total | 61 | 37 | 26 | 124 |
| Artificial tetra- and octo-ploid | | | | |
| *S. Rybinii*, 43/65 ..... | 51 | 20 | 26 | 97 |
| » *acaule* 42/232—1 ..... | 12 | 12 | 8 | 32 |
| Total | 63 | 32 | 34 | 129 |
| Grand total | 124 | 69 | 60 | 253 |
| Per cent. | 49,0 | 27,3 | 23,7 | 100,0 |
| *Lycopersicum* | | | | |
| Artificial tetraploid | | | | |
| *L. esculentum* (UPCOTT, 1935) .... | 67 | 99 | 27 | 193 |
| Per cent. | 34,7 | 51,3 | 14,0 | 100,0 |

should also be pointed out that the tetraploids investigated by the present writer were selected with regard to high fertility, which might decrease the chance of finding interchange heterozygotes.

### e. Types of quadrivalents in *Solanum* and *Lycopersicum*.

All possible types of quadrivalents have been observed with the exception of the types Nos. 19 and 20 of DARLINGTON (1937, Fig. 40). Quadrivalents with multiple chiasmata (DARLINGTON's types Nos. 12, 13, 14, 15, 16 and 18) are, however, less frequent than chain- and ring-quadrivalents. In Table 10 the frequencies of these categories of quadrivalents are given.

The homogeneity of the frequencies between and within different categories of plants has been tested with the aid of the chi-square method. The results of these tests are given below.

*Test of the homogeneity of frequencies of different types of quadrivalents.*

| | Df | $\chi^2$ |
|---|---|---|
| Within *Solanum* | | |
| Between natural plants | 4 | 0,637 |
| Between artificial plants | 2 | 3,914 |
| Between totals of natural and artificial plants | 2 | 1,348 |
| Between *Lycopersicum* and grand total of *Solanum* | 2 | 27,307*** |

There is no significant heterogeneity within *Solanum*, and the grand total may therefore represent the frequency distribution of different types of quadrivalents. Between *Lycopersicum esculentum* and *Solanum* there is a highly significant heterogeneity. In the former species symmetrically arranged quadrivalents are more common than in *Solanum*. This is probably due to the predominance of chromosomes with median primary constrictions in *Lycopersicum*. In *Solanum* a submedian and a subterminal position of the primary constriction are probably the most common.

As to the ring-quadrivalents in *Solanum*, the observations at I—M showed that these quadrivalents were nearly always arranged in non-zigzag form (cf. Figs. 82, 83 and 96). In *Datura* a zigzag arrangement is most common (BELLING and BLAKESLEE, 1924).

### f. Chromosome distribution at II—M.

The distribution of chromosomes between the II—M plates has been investigated in three plants. The chromosome number was counted in 50 plates of each plant investigated, those PMC's being chosen in which one of the two II—M plates could be observed in polar view. The results are given in Table 11.

TABLE 11. *Chromosome numbers counted in II—M plates of three tetraploid Solanum plants.*

| Plants | No. of plates with n = | | | | | | | | No. of plates balanced | No. of plates unbalanced | Percentage balanced |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | | | |
| *S. tuberosum*, Deodara | | | 2 | 12 | 26 | 7 | 3 | | 26 | 24 | 52 |
| » *andigenum*, 36/213 | | | 3 | 10 | 33 | 3 | 1 | | 33 | 17 | 66 |
| » *Rybinii*, 43/65 .... | 2 | 3 | 5 | 8 | 16 | 13 | 2 | 1 | 16 | 34 | 32 |

The numbers of balanced and unbalanced plates of the plants, or more correctly slides, of Table 11, have been compared by the chi-square method (cf. BONNIER and TEDIN, 1940, p. 224). Between *S. tuberosum*, Deodara, and *S. andigenum*, 36/213, there is no heterogeneity. The comparisons *S. Rybinii*, 43/65—*S. tuberosum*, Deodara, and *S. Rybinii*, 43/65—*S. andigenum*, 36/213, both show significant heterogeneity (Df = 1, $\chi^2 = 4{,}105^*$ and $11{,}565^{***}$). This indicates that the frequency of multivalents is higher in *S. Rybinii*, 43/65, than in the natural tetraploids. For comparison it should be mentioned that determinations of the percentage of balanced plates in the tetraploid hybrid *S. chacoense* × *S. tuberosum* vary between 49 and 66 (OPPENHEIMER, 1933; PROPACH, 1938 a), whereas the corresponding determinations in autotetraploid *Lycopersicum esculentum* vary between 63 and 81 per cent (UPCOTT, 1935). The higher values in *Lycopersicum* may be due to a more symmetrical arrangement of the multivalents.

A comparison between the percentage of balanced II—M plates and the percentage of good pollen shows that some of the apparently good pollen must have an unbalanced chromosome number. In trisomic *Lycopersicum* certain extra chromosomes are transmitted through the pollen (LESLEY, 1928), which shows that here unbalanced pollen may be good.

### g. Summing up.

The cytological investigations described in this Chapter have given the following main results:

(1) The chromosomes of *S. acaule* are smaller than those of *S. tuberosum* and *S. Rybinii.*

(2) The number of SAT-chromosomes in the somatic chromosome complement is four in *S. acaule*, two in *S. tuberosum*, and four in artificial autotetraploids of *S. Rybinii.*

(3) When chilled the somatic chromosomes of *Solanum* show supercontraction. The small chromosomes of *S. acaule* contract to a more or less spherical shape. These contracted chromosomes are secondarily associated.

(4) *S. acaule* is characterized by regular bivalent formation. In artificial octoploid *S. acaule* higher primary associations than quadrivalents are not formed.

(5) Occasionally other chromosomes than the SAT-chromosomes acted as nucleolus organizers.

(6) Two kinds of aberrant meiotic behaviour that are common in *S. tuberosum* have been found in autotetraploid *S. Rybinii.* Aberrations of this kind need not indicate hybridity.

(7) There was a positive correlation between the number of SAT-chromosomes of the somatic chromosome complement and the number of nucleoli occurring in the tetrad nuclei.

(8) Multivalents were found in plants of *S. tuberosum* and *S. andigenum*, though not of a higher order than quadrivalents. In an artificial autotetraploid plant of *S. Rybinii* the frequency of multivalents was higher than in these natural tetraploids. The chromosome distribution at II—M also indicates a higher proportion of multivalents in the latter plant.

(9) There was no significant difference in the multivalent frequency between an autotetraploid plant of *S. Rybinii* investigated by the present writer and the quadrivalent frequency of an autotetraploid plant of *Lycopersicum esculentum* investigated by UPCOTT (1935).

(10) The frequency of different types of quadrivalents is not the same in *Lycopersicum esculentum* (UPCOTT, 1935) as in the plants of *Solanum* investigated here.

## D. MORPHOLOGY AND ANATOMY.

### a. Pollen and stomata.

The morphology of *Solanum* pollen has been described by V. KESSELER (1930). The pollen grains are almost round (cf. RYBIN, 1930, Plate X). In the diploids investigated by the author the grains were characterized

by three germination pores. This was also the case with the tetraploid *S. acaule*, whereas in *S. tuberosum* and *S. andigenum* as well as in all the artificial polyploids studied four-pored grains were common. In *S. nigrum* and *Lycopersicum esculentum* WINKLER (1916) and JØRGENSEN (1928 a) have found the same effect of chromosome doubling on the number of germination pores.

In each of seventeen *Solanum* plants the diameters of 100 pollen grains were measured with the aid of an eyepiece micrometer. The pollen was examined in a mixture of glycerine and aceto-carmine and only apparently good grains were measured. These plants belonged to five different groups, namely:

(1) Diploid *S. Rybinii* (Nos. 43/8—1, 43/8—28 and 43/8—37).

(2) Artificial tetraploid *S. Rybinii* (Nos. 43/65, 43/67, 43/68, 43/71 and 43/72).

(3) Natural tetraploids of the group *Tuberosa* (S. *tuberosum*, Deodara and 36/209, and *S. andigenum*, 36/213). For simplicity this group has been called *S. tuberosum*.

(4) Tetraploid *S. acaule* (Nos. 43/231—1, 43/231—2 and 43/231—3).

TABLE 12. *Comparisons of pollen diameters as well as intra-plant variability of pollen size.*

| Comparisons: Plant groups and somatic chromosome numbers | Pollen diameters (M ± m) in $\mu$ and relative size | | Intra-plant variability | | |
|---|---|---|---|---|---|
| | | | Variance and degrees of freedom | | Variance ratio |
| *S. tuberosum* — *S. Rybinii* | 24,3 ± 0,05 | 24,8 ± 0,20 | 3,48 | 6,08 | 1,747*** |
| 2n = 48 2n = 48 | 100 | 102 | 297 | 495 | |
| *S. acaule* — *S. tuberosum* | 22,9 ± 0,38 | 24,3 ± 0,05 | 2,53 | 3,48 | 1,375** |
| 2n = 48 2n = 48 | 100 | 106* | 297 | 297 | |
| *S. Rybinii* — *S. acaule* | 21,4 ± 0,49 | 22,9 ± 0,38 | 2,22 | 2,53 | 1,139 |
| 2n = 24 2n = 48 | 100 | 107 | 297 | 297 | |
| *S. Rybinii* — *S. acaule* | 24,8 ± 0,20 | 27,1 ± 0,51 | 6,08 | 4,98 | 1,223* |
| 2n = 48 2n = 96 | 100 | 109** | 495 | 297 | |
| *S. tuberosum* — *S. acaule* | 24,3 ± 0,05 | 27,1 ± 0,51 | 3,48 | 4,98 | 1,432*** |
| 2n = 48 2n = 96 | 100 | 111** | 297 | 297 | |
| *S. Rybinii* — *S. tuberosum* | 21,4 ± 0,49 | 24,3 ± 0,05 | 2,22 | 3,48 | 1,567*** |
| 2n = 24 2n = 48 | 100 | 114** | 297 | 297 | |
| *S. Rybinii* — *S. Rybinii* | 21,4 ± 0,49 | 24,8 ± 0,20 | 2,22 | 6,08 | 2,742*** |
| 2n = 24 2n = 48 | 100 | 116*** | 297 | 495 | |
| *S. acaule* — *S. acaule* | 22,9 ± 0,38 | 27,1 ± 0,51 | 2,53 | 4,98 | 1,970*** |
| 2n = 48 2n = 96 | 100 | 118*** | 297 | 297 | |

(5) Artificial octoploid *S. acaule* (Nos. 43/232—3, 43/232—13 and 43/232—15).

The main results of this investigation are given in Table 12.

*Statistical.* — The groups have been compared two-and-two, using the method of analysis of variance, and dividing the total variation according to the following scheme:

*Source of variation.*

Total
Inter-group (between group a and b)
Inter-plant intra-group ... a
» » » » ... b
Error 2. Total a + b
Intra-plant intra-group ... a
» » » » ... b
Error 1. Total a + b

Except for the *S. tuberosum* group there were significant differences between the plants within the groups. These differences are probably partly of a genotypical nature, but may also be due to external influences (cf. JOHNSTONE, 1939, p. 301). The significance of the differences between means of groups has been tested by using the inter-plant intra-group variance as error (Error 2). The intra-plant variability has been compared by calculating the ratio between the two parts of the subdivided intra-plant variance.

The standard errors of the means of Table 12 have been calculated on the basis of the subdivided inter-plant intra-group variances. The significances of the relative pollen sizes of Table 12 refer to the inter-group variance ratios: Error 2.

Of the ten possible comparisons between the five different groups the two comparisons *S. Rybinii* ($2n = 48$)—*S. acaule* ($2n = 48$) and *S. Rybinii* ($2n = 24$)—*S. acaule* ($2n = 96$) have been omitted in Table 12, as they are of somewhat less interest than the other comparisons. Also in these two cases the differences were significant.

On doubling the chromosome number the increase of the pollen size is on an average the same in diploid *S. Rybinii* and tetraploid *S. acaule*. The mean pollen diameter of tetraploid *S. acaule* is intermediate between diploid *S. Rybinii* and tetraploid *S. tuberosum*. The difference between diploid *S. Rybinii* and tetraploid *S. acaule* is, however, not significant, probably because of the great inter-plant variability in diploid *S. Rybinii*. The relative size difference between diploid *S. Rybinii* and tetraploid *S. acaule* is about the same as between tetraploid *S. Rybinii* and octoploid *S. acaule*. This latter difference is clearly significant. The group of tetraploid *S. Rybinii* has about the same mean pollen size as the *S. tuberosum* group.

Table 12 further shows that the intra-plant variability of the pollen size is low in diploid *S. Rybinii* and tetraploid *S. acaule*. It is

significantly higher in the *S. tuberosum* group and still higher in the two groups of artificial polyploids.

The length of the guard-cells of the stomata was also determined on a few plants. Strips of epidermis were peeled from the underside near the centre of certain leaflets (cf. p. 9) and mounted in the glycerine aceto-carmine mixture. From each of the plants one leaf of medium size and one large leaf were taken, and 50 stomata per leaf were measured.

The following plants were investigated in this way:
Diploid *S. Rybinii* (Nos. 39/8, 43/8—1, 43/8—16 and 43/8—37).
Artificial tetraploid *S. Rybinii* (Nos. 43/65, 43/68, 43/71 and 43/72).
Tetraploid *S. acaule* (Nos. 42/231—1 and 43/231—1).
Artificial octoploid *S. acaule* (Nos. 42/231—1 and 43/232—15).
The main results of these investigations are given in Table 13.

*Statistical.* — Since the stomata size of two different leaf sizes was studied, the analysis of variance could be made according to alternative schemes. The analysis given below for the comparison of diplo- and tetra-ploid *S. Rybinii* exemplifies the alternative used by the author. As mentioned above, each group consisted here of four different plants.

*Analysis of variance. Comparison between diplo- and tetra-ploid S. Rybinii.*

| Source of variation | | Sum of squares | Df | Mean square |
|---|---|---|---|---|
| Total | | 43876,44 | 799 | Error 2 |
| Inter-group (2n—4n) | | 18480,03 | 1 | 18480,03*** |
| Intra-group | | | | Error 1 |
| inter-plant intra-leaf size | Large leaf size, 2n | 1010,58 | 3 | 336,86*** |
| | Medium » » » | 626,28 | 3 | 208,76*** |
| | Large » » 4n | 3977,33 | 3 | 1325,78*** |
| | Medium » » » | 2537,40 | 3 | 845,80*** |
| | Error 2. Total | 8151,59 | 12 | 679,30*** |
| Inter-leaf size, 2n | | 590,49 | 1 | 590,49*** |
| 4n | | 3152,83 | 1 | 3152,83*** |
| | Total | 3743,32 | 2 | 1871,66*** |
| Intra-leaf | | | | |
| Large leaf size, 2n | | 1915,84 | 196 | 9,77 |
| Medium » » » | | 1993,72 | 196 | 10,17 |
| Large » » 4n | | 4533,82 | 196 | 23,13 |
| Medium » » » | | 5058,12 | 196 | 25,81 |
| | Error 1. Total | 13501,50 | 784 | 17,22 |

A similar comparison has been made between tetraploid and octoploid *S. acaule*. In neither case were the ratios between intra-leaf variances of leaves of medium and large size significant. The considerable differences between the stomata size of leaves of medium and large size are highly significant. Within each group there are significant differences in the mean stomata size between the plants. These differences are greater in the artificial polyploids as compared with the corresponding diplo- and tetra-ploids. This holds good whether the comparisons are made on leaves of medium size or on large leaves. Table 13 therefore refers to the mean of these determinations. The inter-group differences are highly significant and are based on the inter-group variance ratios: Error 2.

TABLE 13. *Comparisons of stoma lengths as well as intra-plant variability of stoma length.*

| Plant groups | Stomatal length in $\mu$ M $\pm$ m | Stomatal length relative | Intra-plant variability Variance | Degrees of freedom | Variance ratio |
|---|---|---|---|---|---|
| *S. Rybinii* (2n = 24) .. | 27,8 $\pm$ 0,917 | 100 | 9,97 | 392 | 2,453*** |
| » » (2n = 48) .. | 38,4 $\pm$ 1,829 | 138 | 24,47 | » | |
| » *acaule* (2n = 48) .. | 31,3 $\pm$ 0,586 | 100 | 6,29 | 196 | 2,001*** |
| » » (2n = 96) .. | 43,7 $\pm$ 0,868 | 140 | 12,58 | » | |

A comparison between Tables 12 and 13 shows that the increase of stomatal length on doubling the chromosome number is greater than the corresponding increase of the pollen diameter. This has also been shown in other *Solanum* species investigated by JOHNSTONE (1939). Table 13 further shows that also in respect of stomatal size the intra-plant variability is larger in the artificial polyploids than in the corresponding diplo- and tetra-ploids. In a poly-haploid twin plant (2n =24) investigated by LAMM (1938) the intra-plant variability of stomata length was greater than in the normal tetraploid twin partner.

The smaller pollen size of *S. acaule* as compared with *S. tuberosum* is of particular interest, since the former species has an allo- and the latter an auto-tetraploid type of meiosis. MÜNTZING (1936) points out that on an average the gigas characters are less marked in experimental allopolyploids than in autopolyploids. HUMPHREY (1937) has shown that *Lycopersicum pimpinellifolium* has smaller pollen grains than *L. esculentum*. It is very probable that in *S. acaule* small plant and small cell size are combined irrespective of polyploid conditions, just as in the example last mentioned. In *Nicotiana* KOSTOFF, GORBATSCHEVA and DIMITROFF (1943) did not find significant differences between the cell size of experimental allo- and auto-polyploids. Thus the cell size of *S. acaule* cannot be used as evidence of allopolyploidy.

### b. Morphological characteristics.

In his paper on autopolyploidy MÜNTZING (1936, pp. 281—283) has given a comparative survey of eight different characteristics. With respect to five of these characteristics the doubling of the number of chromosomes in *S. Rybinii* and *S. acaule* had the following effect:

(1) In *S. Rybinii* the height of the six experimental tetraploids was measured together with ten of their diploid sister-plants. The average plant height of the diploids was $38{,}5 \pm 2{,}531$ cm. and of the tetraploids $31{,}5 \pm 1{,}477$ cm. The difference is not significant.

In the prostrate and rosette-forming *S. acaule* the stem was very short. There was no apparent difference between the tetra- and octo-ploids.

(2) Measurements of the thickness of the leaves and their length-breadth index were taken of some plants of diploid and tetraploid *S. Rybinii* and tetra- and octo-ploid *S. acaule*. The ten lowest leaves were taken on these particular plants, and the measurements were made on certain leaflets (cf. p. 9). For the purpose of determining the thickness fine strips were cut and measured under the microscope with the aid of an eyepiece micrometer. The results, statistically treated in the same way as the measurements of pollen diameters, are given in Table 14.

TABLE 14. *Comparisons of length-breadth indices as well as thickness of leaves.*

| Plant groups | Means of the individual plants | Group means M ± m | Significance Inter-group | Significance Intra-group |
|---|---|---|---|---|
| **Thickness in μ** | | | | |
| *S. Rybinii* (2n = 24) .. | 220 209 203 | 211 ± 5,0 | *** | |
| » » (2n = 48) .. | 297 291 280 271 269 | 282 ± 5,5 | | |
| » *acaule* (2n = 48) .. | 360 329 286 | 325 ± 21,5 | * | * |
| » » (2n = 96) .. | 471 437 392 | 433 ± 22,9 | | * |
| **Length—breadth index** | | | | |
| *S. Rybinii* (2n = 24) .. | 2,37 2,09 1,96 | 2,14 ± 0,121 | * | *** |
| » » (2n = 48) .. | 1,95 1,63 1,62 1,61 1,57 | 1,68 ± 0,070 | | *** |
| » *acaule* (2n = 48) .. | 1,62 1,52 1,48 | 1,54 ± 0,042 | * | * |
| » » (2n = 96) .. | 1,40 1,37 1,30 | 1,36 ± 0,029 | | |

In both species the chromosome doubling has caused an increased thickness of the leaves. In ordinary tetraploid *S. acaule* the leaves are rather thick, and in contrast to most species have two layers of palisade

cells. In the octoploids this number of layers is unaltered, but the cells seem to be larger. The same phenomenon has been found in *Petunia* (HESSE, 1938).

The chromosome doubling was usually accompanied by a decreased value of the leaf index. In *S. Rybinii* there was, however, a considerable intra-group variation of the leaf index. Fig. 104 shows to the left a leaf of the diploid parent plant *S. Rybinii*, 39/8. The three other leaves belong to the tetraploid plants 43/65, 43/71 and 43/68. The last-mentioned plant has rather broad and deformed leaves. The leaves of *S. Rybinii*, 43/71, have an almost diploid shape (length-breadth index = = 1,95).

With respect to the intra-plant variability of these characteristics

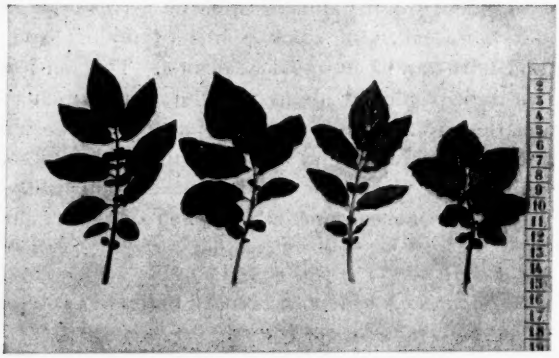


Fig. 104. Leaves of *S. Rybinii*. — From the left to the right leaf of the diploid plant, 39/8, and leaves of the artificial autotetraploid descendants 43/65, 43/71 and 43/68.

no significant differences were found between the original and the chromosome-doubled forms, with the exception of thickness of leaf in *S. acaule*. Here the intra-plant variability was significantly higher in the octoploids.

(3) The radius of ten flowers was measured on each of ten diploid and five tetraploid plants of *S. Rybinii*. There were highly significant differences between the plants within these groups. The mean radius of the diploid group was $19{,}7 \pm 0{,}81$ mm. and of the tetraploid $16{,}2 \pm 1{,}06$ mm. On an average, thus, these tetraploids had smaller flowers than their diploid sister-plants. This difference is also significant. One tetraploid plant was not measured, as the flowers were almost apetalous. The tetraploid plants flowered later than the diploid ones.

In *S. acaule* twenty flowers were collected from tetraploid plants

and twenty from octoploid. The individual plants were thus not studied. The mean radius of the tetraploid flowers was $10{,}3 \pm 0{,}22$ mm. and of the octoploid $12{,}0 \pm 0{,}28$. The differences are highly significant. Also here the flowering of the plants with the higher chromosome number was retarded.

(4) The seed, although not measured, was apparently bigger in the plants with the doubled chromosome numbers. In *S. Rybinii* increased size of the tetraploid seed as compared with diploid has been observed by WEICHSEL (1940). In octoploid *S. acaule* the seed germinated about ten days later than in the tetraploid. It was easy to distinguish the young tetra- and octo-ploid seedlings from each other, since the latter had much broader cotyledons.

On the whole, the effect of chromosome doubling in the autogamous *S. acaule* seems to be more in accordance with the general behaviour among higher plants (cf. KRYTHE and WELLENSIEK, 1942) than in the allogamous *S. Rybinii*. Here the heterozygosity of the diploids is reflected in a considerable variation among the tetraploids.

Finally, it should be mentioned that some of the morphological effects of colchicine treatment are perhaps »dauer» modifications. This might, for instance, be the case with the rough leaf structure of *S. Rybinii*, 43/68 (Fig. 104, furthest to the right).

## 5. INVESTIGATIONS ON A PENTAPLOID SPECIES.

The Svalöf collection of *Solanum* species contained some clones of the cultivated pentaploid species *S. curtilobum*. These clones were badly infected with virus and blight and died entirely away in 1937. My investigations had then been confined to a few observations.

The average pollen fertility was about 40 per cent. The female fertility was quite good, and plenty of seed was obtained, probably from spontaneous crosses with *S. tuberosum* and *S. andigenum*. Attempts to self the plants were less successful, but in 1936 two berries were obtained containing 85 and 83 seeds. From these, 22 and 21 seed plants respectively were obtained. Most of these plants were weak and soon died. Nine of the plants died before root-tips had been collected for the determination of the somatic chromosome numbers. The rest of the plants had the following chromosome numbers:

| | 2n = 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 60 | 62 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of plants . . . . . | 4 | 3 | 5 | 3 | 4 | 4 | 1 | 3 | 6 | 1 | 34 |

This distribution deviates greatly from the expected binominal distribution of plants with chromosome numbers ranging from 48 to 72. However, perhaps the frequency obtained is not representative, since the selfed progeny is small and the chromosome numbers of a comparatively great proportion of the plants have not been determined. Gametic and zygotic elimination are no doubt responsible for these deviations.

Good fixations of the first divisions of meiosis were not obtained. Some PMC's were studied at diakinesis and I—M. The pairing was apparently normal, with mainly bivalents besides univalents, trivalents, quadrivalents and probably also pentavalents. At I—A lagging univalents were observed, some of which were divided.

From the second division good fixations were obtained. In 20 PMC's 27 chromosomes were seen in the plasm outside the II—M plates. This corresponds to an elimination of about 11 per cent of the odd chromosomes $\left(\frac{27 \times 100}{12 \times 20} = 11{,}25\right)$. At the sporad stage, besides tetrads, pentads and hexads were common. Dyads were not observed. The primary purpose of these cytological observations was to verify the absence of gross meiotical aberrations in *S. curtilobum*. In view of the fertility of this species such aberrations, *a priori*, would not be expected.

As previously mentioned (cf. p. 8), the taxonomist assumes that *S. curtilobum* is related to *S. acaule*. One characteristic that supports this assumption is adduced below. On studying anatomical characteristics in *S. acaule*, NASSONOV and YAGER (1933) found that the leaves had two layers of palisade cells. I have found that this is also the case in *S. curtilobum*. *S. tuberosum* has one layer of palisade cells. Hexaploid $F_1$ hybrids between octoploid *S. acaule* and *S. tuberosum* studied by me were also characterized by two layers of palisade cells.

## 6. SECONDARY ASSOCIATION.

### A. INTRODUCTORY NOTES.

The true basic number of the Solanaceous polyploid series has been assumed to be 6 and not 12 (LAWRENCE, 1931 a; MÜNTZING, 1933 a; ELLISON, 1936 a; EMME, 1936 a; KOSTOFF, 1941—1943). This hypothesis is primarily based on studies of secondary association. Before entering upon the descriptive part of this Chapter I should like to recall the following features of this phenomenon.

Secondary association is said to be dependent upon a general

affinity between such homologous or partly homologous chromosomes as for some reason or other (e. g. linear differentiation) are prevented from forming chiasma. This attraction is residual to the primary attraction (DARLINGTON, 1937, p. 497). It starts at pro-metaphase, when the chromosomes are brought close together, appears distinctly at I—M, but is often most marked at II—M. The degree of secondary association follows a normal binominal distribution (CATCHESIDE, 1937). A number of variable conditions affect its expression, such as length of chromosome and constitution of plasm. Quite another interpretation of this phenomenon was given by HEILBORN (1936, 1937). He thought that this association probably has a purely mechanical origin, being a sorting and association effect of the forces of nuclear division upon chromosomes of different size and mass. In this way chromosomes of equal size are associated irrespective of homology, but if homologous chromosomes are present they show association on account of identity in size. Lastly, some cytologists are inclined to interpret this kind of association, at least in *Solanum*, as mainly due to bad fixation resulting in the clumping of the chromosomes (PROPACH, 1937 a).

An analysis of secondary association in diploid and polyploid *Solanum* species has been made by MÜNTZING (1933 a) and PROPACH (1937 a). They found that in diploid varieties a certain proportion of the twelve chromosomes of the II—M plates tended to form secondary groups of two chromosomes each. The average number of chromosomes associated in such groups in the diploids was found by MÜNTZING to be 4,6, by PROPACH 2,8. Among the polyploids secondary groups occurred with proportionately higher numbers of chromosomes in the triploid, tetraploid and hexaploid species. Such higher groups also occurred in the diploids, though less frequently.

According to MÜNTZING, the kind and frequency of association indicate that the original basic number in *Solanum* is 6 and not 12. PROPACH, on the other hand, as mentioned before, believes that the associations are mainly due to bad fixation. He also thinks that the chromosomes associate at random irrespective of a more or less pronounced homology, and that the likelihood of clumping increases parallel with increasing chromosome number. In polyploids the area of the metaphase plate does not increase in proportion to the chromosome number (DARLINGTON, 1937, p. 84). This might facilitate the clumping of the chromosomes as well as their »true» secondary association.

B. ANALYSIS OF SECONDARY ASSOCIATION.

The secondary association in some *Solanum* species has also been studied by me. The slides investigated were rather good. Fine threads connecting the secondarily associated chromosomes, as observed by MÜNTZING (1933 a) and ELLISON (1936 a) in *Solanum* and by GUSTAFSSON (1934) in *Taraxacum*, could not be seen.

In Table 15 the types and frequencies of secondary association in some diploid *Solanum* plants are given. The diploid plant of this table investigated by MÜNTZING (1933 a) was probably *S. stenotomum*. When comparing the observations of different authors allowance must be made for the influence of the subjective factor on the estimation of the degree of association. On the hypothesis of six being the basic chromosome number in *Solanum* the plates have been classed either as normal or as aberrant. In the former class single chromosomes occur together with groups of two (cf. Figs. 10 and 14), in the latter, groups of more

TABLE 15. *Secondary association in diploid Solanum.*

| Configuration. Chromosomes in groups of | | | | | Number of plates | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *S. Rybinii* 43/8—28 | *S. verrucosum* 41/9 | *S. Rybinii* × *S. verrucosum* 43/8 × 9$_7$ | | *S. stenotomum* 37/131 | | MÜNTZING, 1933 a |
| 1 | 2 | 3 | 4 | 5 | I—M | II—M | I—M | II—M | I—M | II—M | II—M |
| 12 | | | | | 5 | 1 | 9 | 10 | 17 | 23 | 5 |
| 10 | 1 | | | | 6 | 6 | 12 | 29 | 18 | 25 | 5 |
| 8 | 2 | | | | 10 | 8 | 9 | 31 | 18 | 18 | 7 |
| 6 | 3 | | | | 7 | 11 | 6 | 9 | 12 | 17 | 6 |
| 4 | 4 | | | | 3 | 2 | 3 | 4 | 7 | | 12 |
| 2 | 5 | | | | | 1 | | 1 | 6 | | 1 |
| 9 | | 1 | | | 3 | 2 | 2 | 3 | 3 | 4 | |
| 8 | | | 1 | | 1 | | | | | | |
| 7 | 1 | 1 | | | 3 | 10 | 2 | 7 | 10 | 7 | 1 |
| 6 | 1 | | 1 | | 3 | 1 | | | 2 | 1 | 1 |
| 6 | | 2 | | | 1 | | 2 | 3 | | 1 | |
| 5 | 2 | 1 | | | 6 | 2 | 1 | 2 | 5 | 3 | 2 |
| 5 | 1 | | | 1 | | | 1 | | 1 | | 1 |
| 5 | | 1 | 1 | | | | 1 | | | | |
| 4 | 2 | | 1 | | | 2 | 1 | 1 | | | |
| 4 | 1 | 2 | | | 2 | 2 | | | 1 | | |
| 3 | 3 | 1 | | | | 2 | 1 | | | 1 | 3 |
| No. of plates: total ..... | | | | | 50 | 50 | 50 | 100 | 100 | 100 | 46 |
| »normal» ... | | | | | 31 | 29 | 39 | 84 | 78 | 83 | 36 |
| Mean No. of groups of two chromosomes in »normal» plates | | | | | 1,90 ± 0,219 | 2,34 ± 0,206 | 1,54 ± 0,197 | 1,65 ± 0,116 | 1,90 ± 0,173 | 1,35 ± 0,121 | 2,50 ± 0,250 |

TABLE 16. *Secondary association in diploid, triploid and tetraploid Solanum.*

| Marginal No. | 2n | Name and field No. | Stage | No. of plates | No. of groups containing the following Nos. of bivalents (I—M) or chromosomes (II—M) | | | | | | Per cent of bivalents (I—M) or chromosomes (II—M) associated in groups of | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1 | 2 | 3 | 4 | 5 | >5 | 1 | 2 | 3 | 4 | >4 |
| 1 | 24 | *S. Rybinii*, 43/8—28 | I—M | 50 | 372 | 79 | 18 | 4 | | | 62,0 | 26,3 | 9,0 | 2,7 | |
| 2 | » | *S. verrucosum*, 41/9 | II—M | » | 338 | 95 | 20 | 3 | | | 56,3 | 31,7 | 10,0 | 2,0 | |
| 3 | » | *S. Ryb.* × *S. verr.* 43/8 × $9_7$ | I—M | » | 414 | 70 | 11 | 2 | 1 | | 69,0 | 23,3 | 5,5 | 1,3 | 0,8 |
| 4 | » | » » » » » | II—M | 100 | 838 | 152 | 18 | 1 | | | 69,8 | 25,3 | 4,5 | 0,3 | |
| 5 | » | *S. stenotomum*, 37/131 | I—M | » | 783 | 172 | 20 | 2 | 1 | | 65,2 | 28,7 | 5,0 | 0,7 | 0,4 |
| 6 | » | » » | II—M | » | 887 | 129 | 17 | 1 | | | 73,9 | 21,5 | 4,2 | 0,3 | |
| 7 | » | » ? (MÜNTZING, 1933 a) | » | 46 | 303 | 106 | 8 | 2 | 1 | | 54,9 | 38,4 | 4,3 | 1,4 | 0,9 |
| 8 | » | *S. chacoense* (PROPACH, 1937 a) | » | 100 | 779 | 141 | 37 | 7 | | | 64,9 | 23,5 | 9,2 | 2,3 | |
| 9 | 36 | *S. chaucha*, 32/8—1 | II—M | 50 | 239 | 147 | 82 | 20 | 7 | 1 | 26,6 | 32,7 | 27,3 | 8,9 | 4,6 |
| 10 | » | » » (MÜNTZING, 1933 a) | » | 40 | 338 | 93 | 49 | 5 | 1 | | 48,6 | 26,7 | 21,1 | 2,9 | 0,7 |
| 11 | » | *S. Commersonii* (v. OLÁH, 1938) | » | 100 | 510 | 245 | 184 | 36 | 10 | 2 | 28,9 | 27,8 | 31,3 | 8,2 | 3,8 |
| 12 | » | *S. acaule* × *S. chacoense* (PROPACH, 1937 a) | » | » | 724 | 281 | 91 | 33 | 2 | | 41,3 | 32,1 | 15,6 | 7,5 | 3,4 |
| 13 | 48 | *S. verrucosum*, 43/54, artificial | II—M | 25 | 122 | 92 | 44 | 23 | 5 | 5 | 20,8 | 31,4 | 22,5 | 15,7 | 9,6 |
| 14 | » | *S. Rybinii*, 43/65, » | » | 50 | 320 | 251 | 79 | 22 | 7 | 1 | 26,9 | 42,3 | 19,9 | 7,4 | 3,4 |
| 15 | » | » » , 41/8—1 b, » | » | 30 | 463 | 93 | 14 | 1 | | | 66,6 | 26,8 | 6,0 | 0,6 | |
| 16 | » | *S. tuberosum*, Deodara. | » | 25 | 154 | 89 | 38 | 22 | 7 | 5 | 25,7 | 29,7 | 19,0 | 14,7 | 11,0 |
| 17 | » | » » (MÜNTZING, 1933 a) | » | » | 187 | 59 | 30 | 19 | 3 | 12 | 32,0 | 20,2 | 15,4 | 13,0 | 19,3 |
| 18 | » | *S. andigenum*, 36/213 | » | 50 | 282 | 162 | 91 | 58 | 13 | 4 | 23,5 | 27,0 | 22,7 | 19,3 | 7,4 |
| 19 | » | *S. acaule*, 42/231—1 | » | 20 | 71 | 60 | 47 | 22 | 8 | 3 | 14,8 | 25,0 | 29,4 | 18,3 | 12,5 |
| 20 | » | *S. ajuscoense* (PROPACH, 1937 a) | » | 100 | 819 | 280 | 125 | 74 | 35 | 27 | 34,1 | 23,3 | 15,6 | 12,3 | 14,6 |

than two chromosomes also occur (cf. Fig. 13). The average numbers of groups of two chromosomes in these so-called normal plates are given in the last lines of Table 15.

Table 16 gives a survey of secondary association in di-, tri- and tetra-ploid plants. In this table the total frequencies of groups of different size classes are given. Single bivalents (I—M) or single chromosomes (II—M) have been listed as »groups of one». To facilitate inter-plant comparisons the degree of secondary association has also been expressed in per cent. The percentages listed, however, do not refer to the numbers of groups but to the numbers of bivalents (I—M) or chromosomes (II—M) associated into the groups of different size classes.

*Statistical.* — The plants of Table 16 have been compared in certain combinations by the chi-square test of homogeneity (cf. BONNIER and TEDIN, 1940, pp. 218—220). When this method is used for comparisons of secondary association it would be most appropriate to work with frequencies of the kind given in Table 15. Especially in the polyploids the number of different configurations that may occur is rather high. Since the expected frequencies should be at least five when the chi-square method is used, an extremely high number of plates must of necessity be analysed. Therefore this method was applied to the numbers of groups of different size classes given in Table 16. This application of the method seems to be permissible, whereas it would be definitely wrong to compare the numbers of chromosomes associated in these groups by the chi-square method. In the diploids groups of more than two chromosomes, in the triploids of more than three, and in the tetraploids of more than four, have been assigned to one class before computing the chi-squares.

Unfortunately, replicate slides of the same plant have not been analysed. Such determinations would have been a valuable basis for statistical analysis.

To begin with, some closely related plants studied by MÜNTZING (1933 a) as well as me will now be compared. Differences in the records pointing in the same direction would suggest subjective influences on the determination of the degree of secondary association. In his diploid *Solanum* (probably *S. stenotomum*) MÜNTZING (1933 a) found a high degree of secondary association, whereas in *S. stenotomum*, 37/131, I found a low degree. This difference is highly significant whether taken from Table 16 ($\chi^2 = 38{,}05^{***}$; $Df = 2$) or from the normal plates of Table 15 ($t = 4{,}14^{***}$; $Df = 117$). In triploid *S. chaucha*, where probably the same clone was studied by both MÜNTZING (1933 a) and me, MÜNTZING found a low and I a high degree of secondary association ($\chi^2 = 51{,}59^{***}$; $Df = 3$). Lastly, the *S. tuberosum* variety Hammersmith analysed by MÜNTZING and the variety Deodara analysed by me, when compared, were found to deviate ($\chi^2 = 10{,}73^{*}$; $Df = 4$). Besides

true differences subjective influences might here account for the divergent results, but it is remarkable that the differences do not point in the same direction.

The next question to be sifted is that of the conditions of secondary association in the diploid *Solanum* plants. Here comparisons have been made between the mean number of groups of two chromosomes in »normal plates» (Table 15) as well as between the numbers of groups of different size given in Table 16. In all cases where a comparison of the kind last mentioned has given a non-significant chi-square, the corresponding comparison of the kind first mentioned has given a non-significant t-value. Also in cases where the differences are significant there is very good agreement between the degrees of the significance of the chi-square and t-values. Henceforward, therefore, only the chi-square values will be mentioned. With reference to the marginal numbers of Table 16 the following chi-squares have been obtained.

*Comparisons of the secondary association of the diploids of Table 16.*

| Comparison between marginal Nos. | $\chi^2$ (Df = 2) | Comparison between marginal Nos. | $\chi^2$ (Df = 2) |
|---|---|---|---|
| Within I—M | | Within II—M | |
| 1—3 | $3{,}93$ | 2—8 | $14{,}42^{***}$ |
| 1—5 | $5{,}66$ | 2—7 | $5{,}28$ |
| 3—5 | $3{,}18$ | 2—6 | $32{,}48^{***}$ |
| Between I—M and II—M | | 2—4 | $20{,}26^{***}$ |
| 3—4 | $1{,}54$ | 4—8 | $11{,}47^{**}$ |
| 5—6 | $11{,}68^{**}$ | 4—7 | $22{,}19^{***}$ |
| | | 4—6 | $3{,}00$ |
| | | 6—8 | $16{,}01^{***}$ |
| | | 6—7 | $38{,}05^{***}$ |
| | | 7—8 | $24{,}06^{***}$ |

Within I—M no heterogeneity has been detected between three different plants. Between the first and second metaphase of the $F_1$ hybrid *S. Rybinii* × *S. verrucosum*, 43/8 × $9_7$, there is no heterogeneity, whereas a corresponding comparison of *S. stenotomum*, 37/131, shows a significant heterogeneity. The main result of the inter-plant comparisons of secondary association during II—M is the detection of considerable heterogeneity.

In the triploids of Table 16 highly significant heterogeneity has been found in five of the six possible comparisons. Only between

*S. chaucha*, 32/8—1, investigated by me, and *S. Commersonii*, investigated by v. OLÁH (1938), there was no heterogeneity ($\chi^2 = 4{,}90$; Df = 3).

Among the tetraploids artificial true autotetraploids and natural tetraploids have been analysed at II—M. In the latter group, because of its regular bivalent pairing, *S. acaule* will here be classified as allopolyploid. *S. ajuscoense*, according to PROPACH (1937 a), is also characterized by bivalent pairing and will be assigned to the so-called allopolyploids, while *S. tuberosum* and *S. andigenum* will be classified here as natural autotetraploids. These classifications, however, are made with reservations as regards the true kind of their tetraploidy, which will be discussed later (cf. p. 100). With reference to the marginal numbers of Table 16 the following chi-squares have been obtained.

*Some comparisons of the secondary association of the tetraploids of Table 16.*

| Classification of the plants (see text above) | Comparison between marginal Nos. | $\chi^2$ (Df = 4) |
|---|---|---|
| **Between artificial autotetraploids** | | |
| normal—normal | 13—14 | $19{,}97^{***}$ |
| » —asynaptic | 14—15 | $163{,}03^{***}$ |
| **Between artificial and natural tetraploids** | | |
| artificial—natural auto. | 13—16 | $3{,}46$ |
| » — » » | 13—18 | $3{,}45$ |
| » — » » | 14—16 | $19{,}18^{***}$ |
| » — » » | 14—18 | $38{,}19^{***}$ |
| » — » allo. | 13—19 | $7{,}83$ |
| » — » » | 14—19 | $52{,}02^{***}$ |
| **Between natural tetraploids** | | |
| auto.—auto. | 16—18 | $3{,}97$ |
| » — » | 16—17 | $10{,}73^{*}$ |
| allo.—allo. | 20—19 | $64{,}12^{***}$ |
| auto.—allo | 16—19 | $17{,}38^{**}$ |
| » — » | 18—19 | $13{,}86^{**}$ |
| » — » | 17—20 | $0{,}62$ |

Among the artificial tetraploids the degree of secondary association was high in *S. verrucosum*, 43/54, somewhat lower in *S. Rybinii*, 43/65, and extremely low in the asynaptic *S. Rybinii*, 41/8—1 b. A typical PMC of the plant last mentioned is shown in Fig. 63. Among the so-called

natural allotetraploids, *S. acaule* had a high and *S. ajuscoense* a low degree of secondary association. In the species last mentioned I have not controlled the statement of PROPACH (1937 a, p. 559) that multivalents do not occur during diakinesis and I—M. There is also a considerable heterogeneity between the so-called natural autotetraploids belonging to the species *S. tuberosum* and *S. andigenum*. No general differences pointing in the same direction have been found in the intergroup comparisons of the representatives of experimental autotetraploids and natural allo- and auto-tetraploids.

### C. DISCUSSION AND CONCLUSIONS.

In the diploid plant analysed by MÜNTZING (1933 a) the type of metaphase plate with four groups of two chromosomes was the most common among the »normal pairing» plates (cf. Table 15). MÜNTZING points out that owing to the small number of plates analysed this maximum is not significant, and this is further corroborated by the present investigation. Generally the maximum frequency of groups of two chromosomes seems to be lower. That great differences may exist between different species must be kept in mind. In the Pomoideae secondary association is much less pronounced in pears than in apples (MOFFETT, 1934). According to DARLINGTON (1937, p. 243), »secondary association can also presumably arise in a diploid from small reduplicated translocations which usually are very general in diploids». Consequently a certain degree of secondary association might also occur in diploids which are not secondary polyploids. To predict a basic number of 6 on the basis of our present knowledge of secondary association in *Solanum* is hardly possible.

Even if 6 is assumed to be the true basic chromosome number in *Solanum*, the occurrence of higher associations than expected on this assumption in the so-called aberrant plates shows the presence of additional forces. As pointed out by CATCHESIDE (1937, p. 368), »these aberrant classes may be due to inferior fixation, mistaken observation, and real structural complexity, especially reduplication, within the complement, in addition to the secondary polyploidy». Because of the heterogeneity of the observations in the diploids of *Solanum* it is difficult to judge the relative importance of the various factors mentioned above as governing secondary association within this genus.

The high degree of secondary association found in *S. acaule* is of special interest, as the opposite situation might have been expected. In comparison with the true artificial autotetraploids and *S. tuberosum*

this species might be looked upon as relatively allopolyploid (cf. p. 100). LAWRENCE (1931 c) assumes that all fertile allopolyploids are the result of the crossing of two related but differentiated species, and hence one would *a priori* expect to find secondary pairing in such polyploids. In *Dahlia coronata*, however, absence of quadrivalents occurs together with a low degree of secondary association, suggesting that this species is strongly allotetraploid. In line with this hypothesis a high degree of secondary association such as found in *S. acaule* would indicate a certain degree of autopolyploidy, which is in agreement with the observations of »differential affinity» in hybrids between *S. acaule* and diploid species (cf. p. 99). The polyploidy of this species is perhaps of the same kind as in the tetraploid *Primula kewensis*. LAWRENCE (1931 a, p. 373) writes: »Thus in the diploid *Primula kewensis*, *verticillata* regularly pair with *floribunda* chromosomes, but in the tetraploid this rarely occurs. Although corresponding chromosome types in the allopolyploid *Primula kewensis* do not normally pair, they will have a general affinity for one another resulting in secondary association». In *S. acaule*, of course, other factors may contribute to the relatively high degree of secondary association, e. g. the small size of the chromosomes. This is perhaps the explanation of the high degree of secondary association between somatic chromosomes in cold treated root-tips of *S. acaule*.

The interaction between primary pairing, differential affinity and secondary association is of special interest in the polyploid *Solanum* species, where all these phenomena occur. LAWRENCE (1931 b) assumes that the stronger secondary association acts between chromosomes which, although identical, have failed to form chiasmata in multivalent primary associations. ELLISON (1936 b), on the strength of his observations of secondary association in *Solanum nigrum* ($2n = 72$) and *S. nitidibaccatum* ($2n = 24$), has suggested that this kind of association appears when, collaterally with a satisfactory primary pairing, there is an unsatisfactory pairing, which would present itself in the haploid. In the diploid *S. nitidibaccatum* he reports lack of secondary association, and so far there is no agreement with the conditions in the diploid species of the section *Tuberarium*. It seems, however, not improbable that the secondary association in the polyploids is partly of the kind suggested by ELLISON, partly of the same kind as in the diploid *Solanum* species of the section *Tuberarium*.

The low degree of secondary association in asynaptic artificial autotetraploid *S. Rybinii*, 41/8—1 b, is rather interesting. It indicates that primary and secondary association may depend on the same forces.

It also indicates that clumping due to bad fixation cannot entirely explain the occurrence of secondary association.

A comparable case of reduced secondary association has been described by DARLINGTON (1937, p. 498): »The B chromosomes of *Zea Mays*, which are inert and over-condensed at metaphase, show by their frequent torsion pairing a reduced primary attraction and by their complete absence of juxtaposition at mitosis a reduced secondary association».

Since the chiasma frequencies of the polyploid *Solanum* plants of Table 16 have not been determined, it is difficult to estimate the correlation between the degree of primary and secondary association. Comparisons between species, as mentioned before, are dangerous, and in order to investigate a possible correlation between primary and secondary association various types of the same species would afford a better material.

Secondary association in *Solanum* seems to be due to a real attraction, probably caused by the same forces as give rise to the primary pairing. A certain degree of clumping due to bad fixation is not excluded, but that this kind of clumping could entirely explain the occurrence of secondary association in good slides seems to be incredible.

It is very likely that the original basic chromosome number in *Solanum* is less than 12, and that the species of this genus are secondary polyploids. The observations of secondary association, however, do not definitely prove this hypothesis. Furthermore, duplication may occur without altering the original basic chromosome number, which would account for a certain degree of secondary association. In any case, the results of analysis of secondary association in *Solanum* are too heterogeneous to prove that the original basic chromosome number of this genus is 6.

## 7. INVESTIGATIONS ON 3x × 2x AND 3x × 4x HYBRIDS.

### A. THE 3x × 2x HYBRIDS.

#### a. Somatic chromosome numbers, morphology and fertility.

In the summers of 1933, 1934 and 1935 plants of the triploid *S. chaucha*, 32/8—1, grafted on tomato were pollinated with pollen from two different highly fertile plants of the diploid species *S. stenotomum*. Four berries were obtained containing 13—21 seeds. Altogether

64 seeds were obtained, from which 23 hybrid plants were raised with the following somatic chromosome numbers:

| 2n | 24 | 25 | 26 | 27 | 28 | 29 |
|---|---|---|---|---|---|---|
| No. of plants ..... | 3 | 10 | 5 | 3 | 1 | 1 |

As might be expected, this progeny showed very great morphological variation. The plants with extra chromosomes were all more or less aberrant in leaf morphology and other characteristics. Some of these plants were dwarfed, and none were as vigorous as their sibs with 24 chromosomes. With one exception plants having more than 25 chromosomes did not flower. This was also the case with three of the trisomic plants. The pollen fertility of three hybrids with 24 chromosomes was 72, 94 and 96 per cent. In the seven flowering trisomic plants the pollen fertility was 0, 0, 5, 10, 34, 39 and 48 per cent. The only double trisomic type that flowered had a pollen fertility of 19 per cent. This plant (field No. 35/2—1), contrary to its trisomic sibs, spontaneously produced a fruit, which, however, dropped before maturity.

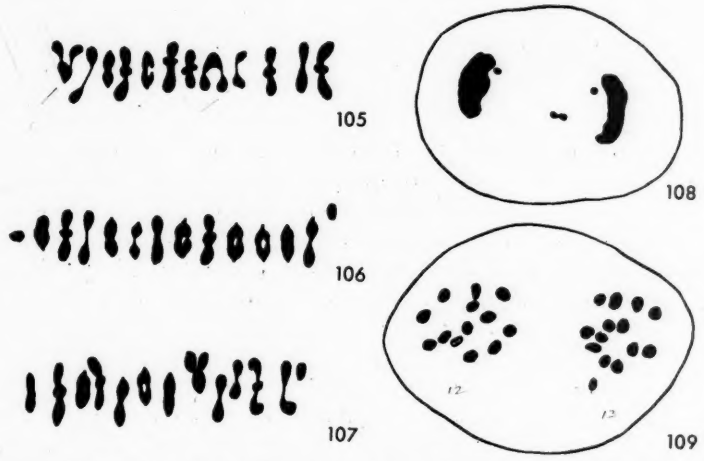
### b. Meiotical investigations.

The meiosis of only the double trisomic plant 35/2—1 will be briefly described here. 100 I—M plates of this plant were analysed in side view. In 18 plates two trivalents (cf. Fig. 105) were observed, in 49 one trivalent plus one univalent (cf. Fig. 107), and in 33 plates two univalents (cf. Fig. 106). This gives a mean number of $1{,}15 \pm 0{,}07$ univalents per cell. The two extra chromosomes were never paired with each other.

If the average pairing tendency of the twelve odd chromosomes in the triploid mother plant should be the same as for these two chromosomes, a mean number of $6{,}9 \pm 0{,}35$ univalents per cell would be expected in the triploid. The mean number of univalents found in 25 PMC's of *S. chaucha* was actually $4{,}1 \pm 0{,}45$ (cf. Table 5), which is significantly lower. In triploid tomato UPCOTT (1935) found a mean number of 7,1 univalents per cell. It is by no means sure that the average pairing tendency of these two chromosomes would be the same as that of all the odd chromosomes of the triploid together. Still, I have made this calculation, as the observations of metaphase pairing in this double trisomic plant are more reliable than those made in *S. chaucha*. [The standard error $\pm 0{,}35$ of the mean 6,9 has been obtained from the expected frequency of univalents $= 25(0{,}575 + 0{,}425)^{12}$.]

Division of the univalents at I—A was sometimes observed (cf. Fig. 108) and this observation could be checked at II—M (cf. Fig. 109). Eliminated univalents were frequently observed at interphase and later

stages. At the sporad stage, besides tetrads, dyads and pentads were observed.




Figs. 105—109. Meiosis in the PMC's of a double trisomic $F_1$ hybrid of *S. chaucha* × *S. stenotomum*. — Figs. 105—106, I—M groups (separately drawn) showing varying pairing conditions of the two odd chromosomes; Fig. 108, I—T showing one lagging, undivided and one divided univalent; Fig. 109, II—A plates showing the chromosome distribution 12 1/2—13 1/2. — × 2550.

### B. THE 3x × 4x HYBRIDS.

#### a. Somatic chromosome numbers.

In the summer of 1934 *S. chaucha*, 32/8—1, was crossed with the *S. tuberosum* varieties Gammal röd and Deodara. According to BUKASOV (1933, p. 129) this cross is not successful, and this was also my experience in experiments with ordinary plants of the triploid, which were grown in the field. From grafted triploid plants grown in the greenhouse, however, hybrid seed was obtained. From the cross with Gammal röd as male parent (cross A) one fruit with 35 seeds was obtained, and from the cross with Deodara (cross B) one fruit with 85 seeds. All attempts to use *S. chaucha* as male parent either in selfings or in crosses with diploid and tetraploid species failed.

The seed was rather heterogeneous in size, and a large portion did not germinate. The chromosome numbers of the plants raised from these progenies are given in Table 17.

The deviation from the expected random distribution of chromosome numbers between 36 and 48 with a maximum at 42 is very great

TABLE 17. *Chromosome numbers in $F_1$ of crosses between S. chaucha, 32/8—1 (2n = 36) and S. tuberosum (2n = 48).*

| Somatic chromosome No. | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A. 32/8—1 × Gammal röd ..... | | | 1 | 1 | 2 | | 6 | 7 | | 2 | 19 |
| B. » × Deodara ..... | 1 | 1 | 1 | 6 | 5 | 4 | 11 | 12 | 6 | 5 | 52 |
| Total | 1 | 1 | 2 | 7 | 7 | 4 | 17 | 19 | 6 | 7 | 71 |

indeed. Since *S. chaucha* has not been embryologically investigated, it is impossible to tell which are the main causes of this deviation. In triploid *Datura* there is a strong elimination of unbalanced zygotes and in many fertilized ovules the pro-embryo and endosperm desintegrate soon after fertilization (SATINA, BLAKESLEE and AVERY, 1938). Not only zygotic but also gametic elimination may be responsible for the results obtained in these *S. chaucha* × *S. tuberosum* crosses.

### b. Pollen fertility and vigour.

The pollen fertility of the parent species and varieties has been reported earlier. Twelve of the $F_1$ hybrids did not flower. Proportion-

TABLE 18. *Correlation between chromosome number and pollen fertility as well as chromosome number and plant vigour in $F_1$ S. chaucha (2n = 36) × S. tuberosum (2n = 48).*

(F = per cent good pollen; V = mark for vigour.)

| 2n | 39—42 | | | | 43—44 | | | | 45—46 | | | | | | 47—48 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cross | A | | B | | A | | B | | A | | B | | B | | A | | B | |
| | F | V | F | V | F | V | F | V | F | V | F | V | F | V | F | V | F | V |
| | *2n = 39* | | | | *2n = 43* | | | | *2n = 45* | | | | | | *2n = 47* | | | |
| Pollen | | | 0 | 2 | 42 | 2 | 5 | 4 | 75 | 4 | 83 | 4 | 42 | 3 | | | 65 | 5 |
| fertility | *2n = 40* | | | | 48 | 1 | 0 | 3 | 10 | 4 | 70 | 4 | 57 | 2 | | | 86 | 4 |
| (F) | | | 20 | 2 | 31 | 1 | 32 | 2 | 9 | 1 | 61 | 4 | 48 | 1 | | | 58 | 4 |
| and | *2n = 41* | | | | *2n = 44* | | | | 0 | 1 | 31 | 4 | 47 | 1 | | | 46 | 4 |
| plant | 0 | 1 | | | | | 69 | 4 | | | 49 | 3 | 47 | 1 | | | 40 | 4 |
| vigour | *2n = 42* | | | | | | 0 | 1 | | | | | 41 | 1 | *2n = 48* | | | |
| (V) | 1 | 3 | 4 | 4 | | | | | *2n = 46* | | | | | | 80 | 5 | 74 | 5 |
| of the | | | 44 | 2 | | | | | 66 | 5 | 78 | 5 | 68 | 3 | 58 | 4 | 72 | 4 |
| indivi- | | | 0 | 1 | | | | | 64 | 5 | 68 | 4 | 59 | 2 | | | 65 | 4 |
| dual | | | 0 | 1 | | | | | 48 | 5 | 64 | 4 | 67 | 1 | | | 64 | 4 |
| plants | | | | | | | | | 82 | 4 | 84 | 3 | 61 | 1 | | | 80 | 1 |
| | | | | | | | | | 79 | 4 | 80 | 3 | | | | | | |
| | | | | | | | | | 64 | 3 | 72 | 3 | | | | | | |
| | F | | V | | F | | V | | F | | | V | | | F | | V | |
| Mean | 8,6 | | 2,00 | | 28,4 | | 2,25 | | 57,2 | | | 3,00 | | | 65,7 | | 4,00 | |

ally the non-flowering plants were the most common among the hybrids with a somatic chromosome number less than 45. It should also be mentioned that after open pollination fruits were harvested on seventeen plants representing the chromosome numbers 45—48, whereas plants with fewer than 45 chromosomes did not set seed spontaneously. The pollen fertility of the flowering hybrids was determined in the summers of 1935 and 1936. Averages of these determinations are given in Table 18.

*Statistical.* — The figures for pollen fertility given in Table 18 have been analysed by the method of covariance devised by BONNIER and TEDIN (1940, pp. 156—159) as follows:

| Source of variation | Df | Sum of squares | Mean square |
|---|---|---|---|
| Deviation, means of lots (cross A and B) from the average intra-lot regression line ..... | 1 | 193,73 | 193,73 |
| Deviation within lots from intra-lot regression lines .. | 55 | 20804,67 | 378,27 |
| Inter-lot deviation between regression coefficients .... | 1 | 15,86 | 15,86 |
| Deviation from parallel intra-lot regression lines (error) | 56 | 20820,53 | 371,80 |
| Total | 57 | 21014,26 | |

This analysis shows that there are significant differences either between the mean pollen fertility of the two crosses or between the two corresponding regression coefficients (10,12 and 9,53). The statistical analysis has therefore been completed by the following test:

*Test of deviation from linear regression between chromosome number and pollen fertility referring to the 59 plants of Table 18.*

| Source of variation | Df | Sum of squares | Mean square |
|---|---|---|---|
| Total ..... | 58 | 44264,54 | |
| Inter-chromosome numbers ..... | 9 | 27206,87 | 3022,99*** |
| Intra- » » (error) ... ..... | 49 | 17057,67 | 348,12 |
| Linear regression ..... | 1 | 23250,08 | 23250,08*** |
| Deviation from linear regression ..... | 8 | 3956,79 | 494,60 |

The statistical analysis has shown that there are no significant differences in pollen fertility between the two progenies. Further, there is a highly significant regression between somatic chromosome number and pollen fertility. The average fertility increases with increasing chromosome number.

The $F_1$ progenies between *S. chaucha* and *S. tuberosum* showed a very great variation in morphological characteristics and plant vigour. Some of the hybrids, just as their triploid mother, had a poor capacity

for tuber production, others in this respect approached more closely to *S. tuberosum*. As to the absolute vigour of these hybrids nothing can be said, owing to the fact that some of the plants were certainly not photo-periodically adapted to the light conditions of South Sweden (cf. JOHNSSON, 1940, pp. 345—346). Still, the vigour of the plants was estimated in 1936, marks being awarded according to a scale of five grades. The apparently most vigorous plants were awarded the mark 5, the weakest the mark 1. Within each class of vigour the morphological variation of one and the same characteristic, e. g. plant height, was considerable. There were weak plants that were high and slender, and vigorous plants that were comparatively short but robust, and so on. The results of these evaluations of vigour, which are given in Table 18, strongly indicate a positive correlation between chromosome number and plant vigour.

*Statistical.* — Statistical methods for the treatment of subjectively classified data have been given by WAHLUND (1931). Without certain corrections analysis of variance should here only be made if the errors of the estimations are normally distributed. In the present case the differences between the group means of Table 18 and the individual estimations of vigour within the groups (e. g. groups $2n = 43—44$; 2,25—2, 2,25—1, . . . 2,25—4, 2,25—1) give a significantly skew distribution. In view of the uncertainty of the estimations of plant vigour I have not thought it worth while to carry out a statistical analysis. Moreover, an analysis would necessitate the application of the more laborious method of WAHLUND.

### c. Meiotical investigations.

Meiosis was studied in twenty $F_1$ hybrids representing all the different chromosome numbers. The slides of the first division were, however, not good enough to permit an analysis of the configurations. The pairing seemed to be normal, and no great aberrations such as asynapsis or inhibition of the second division occurred even in the sterile plants. During I—A lagging univalents frequently occurred and were sometimes divided at this stage. Eliminated univalents were often observed at II—M.

### d. Discussion.

The phenomenon of secondary polyploidy has been touched upon in the Chapter on secondary association. Another criterion of secondary polyploidy is the occurrence of secondary balance in the triploid progenies (cf. MOFFETT, 1931). In back-crosses of the triploid hybrid *Nicotiana paniculata* ($2n = 24$) $\times$ *N. rustica* ($2n = 48$) to its parents, LAMMERTS (1929) found random distribution of the extra chromosomes. Among the $3x \times 2x$ progenies plants with 30 chromosomes were the

most common, among the 3x × 4x progenies plants with 42 chromosomes. This behaviour is not common in 3x × 2x progenies of plants with low basic chromosome numbers (cf. LEVAN, 1942 b, p. 376). The instance mentioned above in *Nicotiana* has been interpreted by DARLINGTON (1937, pp. 326—327) as being due to a secondary balance between the primary basic number of 6 and the secondary basic number of 12. On the other hand, similar crosses in other Solanaceous plants do not indicate the existence of a secondary balance of this kind. In backcrosses of the triploid hybrid *Nicotiana sylvestris* (2n=24) × *N. tabacum* (2n = 48) to its diploid parent the distribution of chromosome numbers is discarded in the diploid direction, trisomic and double trisomic types being the most common (GOODSPEED and CLAUSEN, 1922). Such skewness as against the expected random distribution is also characteristic of 3x × 2x crosses in *Lycopersicum esculentum* (LESLEY, 1928), *Datura stramonium* (cf. LEVAN, 1942 b) and *Nicotiana sylvestris* (GOODSPEED and AVERY, 1939). Among these species there are differences in the sensibility to change of the numerical balance. Nor do my 3x × 2x and 3x × 4x crosses in *Solanum* indicate the existence of a secondary balance in favour of the assumed primary basic number 6.

In the $F_1$ progeny of *S. Commersonii* (2n = 36) × *S. Henryi* (2n = 24) v. OLÁH (1938) found a distribution of chromosome numbers similar to that among the *S. chaucha* × *S. stenotomum* hybrids of the present investigation. The former two species are taxonomically closely related. In the $F_1$ progeny between *S. Commersonii* and the taxonomically somewhat more remotely related diploid species, *S. chacoense* hybrids with 32—34 chromosomes were obtained. v. OLÁH (1938) points out that a viable zygote may be obtained from the union of a haploid chromosome set of *S. Henryi* with 12—15 chromosomes of *S. Commersonii*, while the haploid chromosome set of *S. chacoense* requires 20—22 chromosomes of *S. Commersonii*. He thinks that the degree of genotypical relationship could be inferred from the chromosome distribution in the $F_1$ of such 3x × 2x crosses. If this hypothesis is valid, *S. chaucha* and *S. stenotomum* should be closely related. The bearing of v. OLÁH's hypothesis on 3x × 4x progenies will be discussed later.

Certain tetraploid *Solanum* species, e. g. *S. tuberosum* and *S. andigenum*, frequently form numerically unbalanced gametes. Aneuploid varieties of these species have not yet been found. To a certain degree this might be explained by the decreased fertility and vigour of aneuploid types, which decrease has been demonstrated in the present paper. In the next Chapter we shall see, however, that the effect of

chromosomes in excess of 48 seems to be less deleterious. Any exact determination of such effects would require more extensive field trials.

## 8. INVESTIGATIONS ON 5x × 4x AND 4x × 5x HYBRIDS.

### A. PREVIOUS INVESTIGATIONS.

The result of reciprocal crosses between *S. curtilobum* and *S. tuberosum* has been published earlier by LAMM (1941). It was found that the pentaploid *S. curtilobum*, 36/218, could be easily crossed with *S. tuberosum*, 36/209, whereby fertile hybrids with intermediate chromosome numbers were raised. The reciprocal cross was difficult to carry out, but was successful on grafting the mother plant *S. tuberosum*, 36/209, on tomato. The hybrids thus obtained, which also had intermediate chromosome numbers, were completely male-sterile and their PMC's showed an aberrant course of meiosis. This dissimilar behaviour of the reciprocal hybrids was assumed to be due either to cytoplasmic inheritance or to maternal effects. In this Chapter some supplementary results obtained by further investigations of these hybrids will be given.

### B. SOMATIC CHROMOSOME NUMBERS, PLANT VIGOUR AND FERTILITY.

*S. curtilobum*, 36/218, was crossed in the summer of 1936 with *S. andigenum*, 36/213. There was plenty of seed and the following year 100 $F_1$ hybrids were raised. The distribution of somatic chromosome numbers of this 5x × 4x cross is given in Table 19, which also indicates the corresponding distribution of the previously published 4x × 5x cross between *S. tuberosum*, 36/209, and *S. curtilobum*, 36/218 (LAMM, 1941). The chi-square of Table 19 computed on 6 Df shows that there is no significant heterogeneity between these two distributions.

In the PMC's of *S. curtilobum* about 11 per cent of eliminated odd chromosomes were observed at II—M (cf. p. 66). The total elimination may be higher and is best studied at the sporad stage or during the first pollen grain division (cf. CLAUSEN, 1928). In the absence of such determinations the expected chromosome distributions of Table 19 have been calculated on the basis of an elimination of 11 per cent. In the cross *S. tuberosum* (36/209) × *S. curtilobum* (36/218) the chi-square test shows a good agreement between the observed and expected distribution. In the other cross of Table 19 there is a significant deviation, which if anything indicates a lower degree of chromosome elimination than in the 4x × 5x cross mentioned above. This is somewhat puzzling, since *S. curtilobum* was the female partner in this cross. In triploid *Datura* the chromosome elimination of the EMC's is much greater than that of the PMC's (SATINA and BLAKESLEE, 1937 b). The above suggestions, however, are of little significance, since the degree of chromosome

TABLE 19. *Chi-square tests of the distributions of chromosome numbers in 5x × 4x and 4x × 5x* crosses.

| Species<br>Field No.<br>2n → | *S. curtilobum* × *S. andigenum*<br>36/218<br>60 | | *S. tuberosum* × *S. curtilobum*<br>36/209<br>48 | |
|---|---|---|---|---|
| ↓ | Expected | Found | Found | Expected |
| 48 | 0,09 | 3 | 1 | 0,04 |
| 49 | 0,82 | 3 | | 0,42 |
| 50 | 3,62 | 5 | 4 | 1,85 |
| 51 | 9,68 | 5 | 5 | 4,94 |
| 52 | 17,47 | 10 | 8 | 8,91 |
| 53 | 22,42 | 15 | 7 | 11,43 |
| 54 | 20,97 | 23 | 10 | 10,69 |
| 55 | 14,41 | 16 | 7 | 7,35 |
| 56 | 7,22 | 15 | 6 | 3,68 |
| 57 | 2,58 | 3 | 2 | 1,31 |
| 58 | 0,62 | 2 | | 0,32 |
| 59 | 0,10 | | 1 | 0,05 |
| 60 | 0,01 | | | 0,00 |
| Total | 100,01 | 100 | 51 | 50,99 |
| | 14,21 | 11 | 10 | 7,25 |
| | 10,53 | 20 | 9 | 5,36 |
| Hypothesis | [2]) $100\,(0{,}555 + 0{,}445)^{12}$ | [1]) No hypothesis | [2]) $51\,(0{,}555 + 0{,}445)^{12}$ | |
| Df | 5 | 6 | 5 | |
| $\chi^2$ | 14,764* | 2,566 | 5,386 | |

elimination during meiosis cannot be estimated from the chromosome distribution of the zygotes. As to these 5x × 4x and 4x × 5x crosses the essential statement is that hybrids with intermediate chromosome numbers (4—8 extra chromosomes) are the most common, in contradistinction to the 3x × 2x and 3x × 4x crosses of the previous Chapter. In the summer of 1937 the pollen fertility and plant vigour of these *S. curtilobum* × *S. andigenum* hybrids were determined in the same way as described earlier (cf. p. 78). Table 20 gives the average results of these determinations.

Of the hybrid plants, 25 did not flower. These generally seem to have been less vigorous than the flowering plants. As regards morphology, the $F_1$ progeny showed considerable variation. The figures in Table 20 do not indicate a correlation between chromosome number and vigour. This is also the case with the pollen fertility, as can be seen

[1] BONNIER and TEDIN, 1940, pp. 218—221.
[2] » » » » » 195—197.

TABLE 20. *Correlation between chromosome number and pollen fertility as well as chromosome number and vigour in $F_1$ hybrids of the 5x × 4x cross S. curtilobum (36/218) × S. andigenum (36/213).*

| 2n | No. of plants | | | Mean marks for vigour | | Mean fertility (flowering plants) |
|---|---|---|---|---|---|---|
| | Non-flowering | Flowering | Total | Non-flowering | Flowering | |
| 48 ..... | | 3 | 3 | | 3,0 | 49,7 |
| 49 ..... | 1 | 2 | 3 | 4,0 | 4,0 | 35,0 |
| 50 ..... | 1 | 4 | 5 | 2,0 | 4,0 | 75,8 |
| 51 ..... | 1 | 4 | 5 | 4,0 | 2,8 | 56,3 |
| 52 ..... | 4 | 6 | 10 | 2,3 | 3,3 | 53,2 |
| 53 ..... | 3 | 12 | 15 | 2,0 | 3,0 | 34,0 |
| 54 ..... | 7 | 16 | 23 | 2,4 | 3,6 | 38,9 |
| 55 ..... | 3 | 13 | 16 | 2,3 | 2,8 | 58,3 |
| 56 ..... | 3 | 12 | 15 | 1,7 | 2,9 | 37,8 |
| 57 ..... | 1 | 2 | 3 | 2,0 | 3,5 | 68,5 |
| 58 ..... | 1 | 1 | 2 | 1,0 | 2,0 | 53,0 |
| Total | 25 | 75 | 100 | | | |
| | | Mean of all the plants | | 2,3 | 3,2 | 46,7 |

from the analysis below, where none of the mean squares are significant when tested against the »error mean square».

*Test of deviation from linear regression between chromosome number and pollen fertility.*

| Source of variation | Df | Sum of squares | Mean square |
|---|---|---|---|
| Total ..... | 74 | 53798,99 | |
| Inter chromosome numbers ..... | 10 | 10876,10 | 1087,61 |
| Intra » » (error) ... | 64 | 42922,89 | 670,67 |
| Linear regression ..... | 1 | 324,21 | 324,21 |
| Deviation from linear regression ..... | 9 | 10551,89 | 1172,43 |

Several of these $F_1$ hybrids spontaneously produced berries.

Of *S. tuberosum* (36/209) × *S. curtilobum* (36/218) only 24 $F_1$ hybrids have been studied (LAMM, 1941). With respect to the distribution of chromosome numbers, pollen fertility and plant vigour, these behaved in the same way as the *S. andigenum* × *S. curtilobum* hybrids described here.

The hybrids of the 4x × 5x cross between *S. tuberosum*, 36/209, and *S. curtilobum*, 36/218, as mentioned before, were completely male-sterile. They also seemed to be rather female-sterile. Every summer since 1937 attempts have been made to back-cross these hybrids with their parent species. Back-crosses with *S. curtilobum* invariably failed.

In 1940 the back-cross of one of the hybrids with *S. tuberosum* succeeded, and in the summer of 1943 two more of these hybrids were successfully back-crossed.

The back-cross first mentioned was performed between an $F_1$ hybrid having the somatic chromosome number 52 and *S. tuberosum*, 36/209. The following chromosome distribution was obtained in the progeny.

| | 2n 48 | 49 | 50 | 51 | 52 | 53 | 54 | Total |
|---|---|---|---|---|---|---|---|---|
| No. of plants observed ... | 17 | 15 | 15 | 11 | 6 | 2 | 1 | 67 |
| » » » expected ... | 4,2 | 16,8 | 25,1 | 16,8 | 4,2 | | | 67,1 |

The expected frequency has been calculated on the basis of random distribution of the four extra chromosomes $[67(0{,}5 + 0{,}5)^4]$. There is a highly significant deviation between *observed* and *expected*, which may be due to the process of elimination of chromosomes, gametes and zygotes discussed earlier. The occurrence of plants with more than 52 chromosomes may be due to non-separation of split univalents during the first or second division. In this back-cross progeny some plants soon died away and some plants did not flower. Of the flowering plants, 34 were completely male-sterile and 10 partially, having between 1 and 25 per cent good pollen. Apparently there was no correlation between chromosome number and fertility.

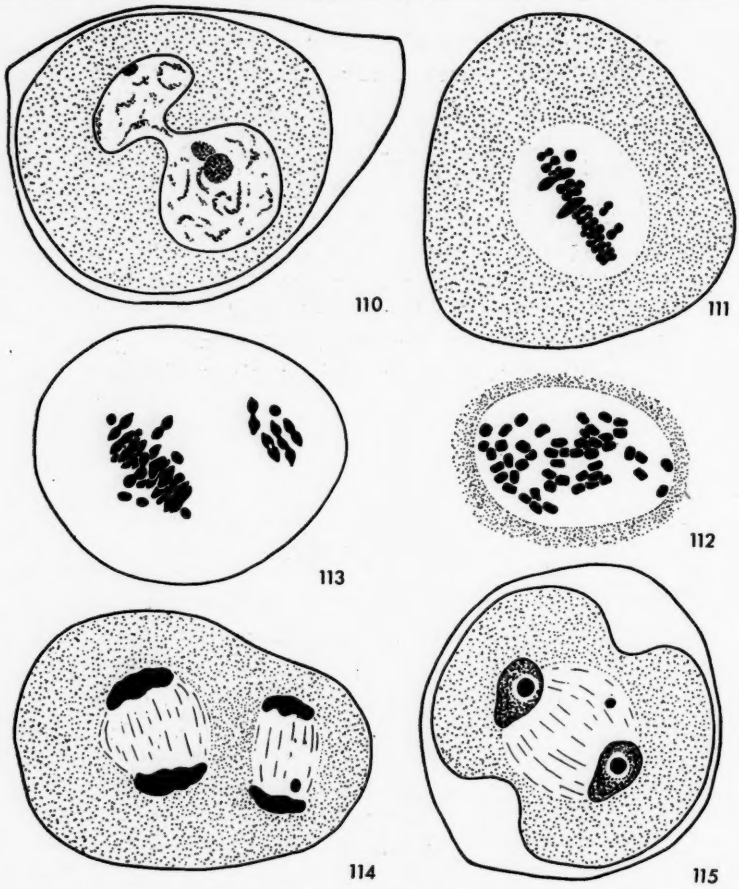
### C. MEIOTICAL INVESTIGATIONS.

Some supplementary facts obtained in the investigations on the male-sterile $F_1$ hybrids of the cross *S. tuberosum* (36/209) $\times$ *S. curtilobum* (36/218) will be given below (cf. LAMM, 1941, pp. 204—205).

In the PMC's of these hybrids, hourglass-like constrictions of the prophase nuclei were frequently observed (Fig. 110). Not uncommonly one or two small additional nuclei detached from the larger nucleus were to be seen. Later stages of these additional nuclei are shown in Figs. 113 and 114. Similar behaviour has been observed by BLEIER (1930, 1931 b) in hybrids between *Triticum* and *Secale* and by DARLINGTON and THOMAS (1937) in *Festuca* $\times$ *Lolium* derivatives. Possibly these irregularities are due to abnormal spindle behaviour at the premeiotic mitosis.

According to an earlier paper of mine (LAMM, 1941), only univalents appear at I—M. Occasionally, however, gemini are found. Pure pseudo-homotypic metaphases have been illustrated earlier (l. c., p. 204). Fig. 111 shows a I—M mainly consisting of regularly arranged dividing

univalents together with a few bivalents. Fig. 112 shows an irregular distribution of undivided univalents, while Fig. 113 shows a PMC of the same plant at I—M with apparently normal pairing of the chromo-




Figs. 110—115. Meiosis in the PMC's of one of the hybrids *S. tuberosum* × *S. curtilobum*, 26—5. — Fig. 110, early prophase with a nucleus resembling an hour-glass; Fig. 111, I—M with a few bivalents and several dividing univalents; Fig. 112, I—A with irregular distribution of undivided univalents; Fig. 113, I—M showing ordinary gemini formation. The chromosomes are distributed between two groups; Fig. 114, same as Fig. 113 at I—T; Fig. 115, formation of a dyad. — × 2550.

somes. As a rule, meiosis takes place in the centre of the PMC, where the cytoplasm is rather refractive as compared with the surrounding

dense cytoplasm. Occasionally there is no such differentiation of the cytoplasm, e. g. in the PMC of Fig. 113. Sometimes pseudo-homotypic division has characterized the PMC's of one anther, and normal pairing

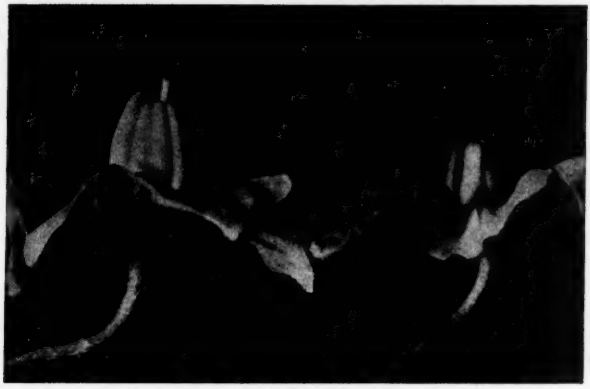


Fig. 116. To the left a flower of $F_1$ *S. curtilobum* × *S. tuberosum*, 21—3, to the right of $F_1$ *S. tuberosum* × *S. curtilobum*, 26—5. The latter flower has reduced anthers.

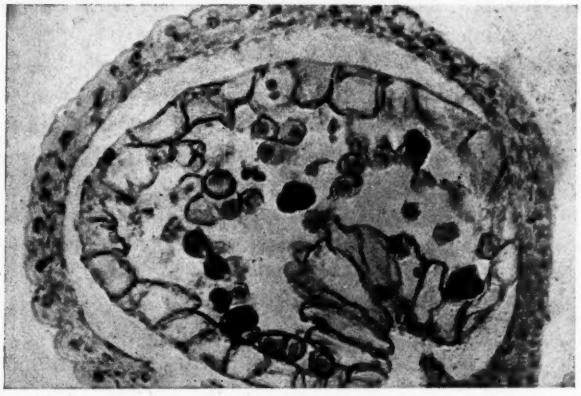


Fig. 117. Section through an anther of the male-sterile $F_1$ hybrid *S. tuberosum* × *S. curtilobum*, 26—5.

the PMC's of another anther of the same flower. Obviously the course of the first division of meiosis is highly influenced by environmental conditions.

Common to all the hybrids investigated is the abortion of the second division. Dyads are occasionally formed (Fig. 115). Fig. 114 shows

I—T in a PMC earlier provided with one large and one small nucleus. Such a configuration might easily be mistaken for a normal II—T. Degeneration of the PMC nucleus as early as prophase has sometimes been observed, but a degeneration of the interphase nuclei is the most common occurrence.

The anthers of these male-sterile hybrids were more or less aborted in comparison with those of the normally fertile reciprocal hybrids. The colour of these aborted anthers varied from pale yellow to light green. Fig. 116 to the left shows a flower of the male-fertile hybrid *S. curtilobum* × *S. tuberosum*, 21—3, to the right one of the male-sterile hybrid *S. tuberosum* × *S. curtilobum*, 26—5. Fig. 117 shows a section through an anther of the plant last mentioned. The cavities of the anthers of this plant are occupied by ingrowing vegetative tissue. This aberration, which has also been met with in certain varieties of *S. tuberosum* (BAYLISS, 1936), was also found in some of the other male-sterile hybrids. In *S. demissum* v. KAUSCHE (1937) found some correlation between aberrant tapetal development and aberrant meiotic behaviour of the PMC's. Slides made by me of anthers from some reciprocal *S. curtilobum* × *S. tuberosum* hybrids were not good enough for comparative investigations of the tapetal development of these plants.

### D. DISCUSSION.

In the preceding Chapter we have seen the deleterious effects of decreasing the somatic chromosome number below 48. A corresponding increase of this chromosome number has apparently little effect on vigour and fertility. The back-cross progeny of a hybrid having 52 chromosomes to its tetraploid mother gave, however, a considerable excess of tetraploid plants. In the crosses studied here extra chromosomes of *S. curtilobum* have been added to a hybrid chromosome complement. A corresponding intra-specific numerical change may have a different effect on fertility and vigour (cf. pp. 108—109).

The cross between *S. tuberosum* (♀) and *S. curtilobum* (♂) has shown that in the species last mentioned numerically unbalanced male gametes are functional. BECKER (1939) assumes that only pollen grains with 24 chromosomes are active in the pentaploid $F_1$ hybrid *S. demissum* × *S. tuberosum*. This author determined the chromosome numbers in a progeny of the $F_1$ hybrid obtained after free flowering. The possibility of accidental back-crossing with *S. tuberosum* was not excluded, and seems rather likely in the light of the present investigation. Differences in the fertility of reciprocal crosses within the section

*Tuberarium* have been found by SALAMAN and LESLEY (1922) on crossing two varieties of *S. tuberosum*, and by IVANOV (1939) on crossing the two tetraploid species *S. Antipoviczii* and *S. tuberosum*. The corresponding case in the present paper is thus not unique but in the matter of details there are differences between all three cases. It was much more difficult to produce the sterile combination *S. tuberosum* × *S. curtilobum* than the reciprocal fertile one. It is, however, not certain that there is a causal connection between the difficulties of crossing and the sterility of the hybrids. In crosses between the diploid *S. chacoense* and the tetraploid *S. tuberosum* the 2x × 4x combination is much easier to realize than the reverse. Owing to the occurrence of unreduced gametes in the diploid tetraploid hybrids are frequently obtained. These are fertile irrespective of the direction of the cross (STELZNER, 1943 a).

Interaction between the same chromosome complement and different cytoplasms resulting in male fertility *versus* male sterility is frequently met with in cases of cytoplasmic inheritance (cf. CORRENS, 1937; SIRKS, 1938). In hybrids between the diploid species *Nicotiana Langsdorffii* and *N. Sanderae* EAST (1932) has given the factorial interpretation of a case of the kind mentioned above. In *Lycopersicum* SCHLÖSSER (1935) has shown a cytoplasmic determination of the osmotic properties, which in their turn act upon the phenotypical effects of certain factors for length. A probable case of maternal effects on the manner of growth in *Datura* has been published by SIRKS (1940). Interactions of these kinds are thus also known in other genera of the Solanaceous plants.

Influence of the plasm on chromosome conjugation in *Oenothera* has been shown by OEHLKERS (1935). This influence was rather sensitive to external conditions. In the *S. tuberosum* × *S. curtilobum* hybrids, too, external influences seem to some extent to govern the course of meiosis. Since meiosis was mainly studied in these hybrids in smears of the PMC's, the influence of the position of these cells relative to the tapetum has not been determined. WIEBALCK (1940) found a correlation between the conditions of chromosome pairing in the PMC's and the distance between these cells and the tapetum. This author has also demonstrated the influence of the osmotic value on chromosome pairing, and these investigations together with those of GREGORY (1940) tend to show that further work on the *S. tuberosum* × *S. curtilobum* hybrids would be productive.

The genetical interpretations of cytoplasmic inheritance in diploids are often somewhat complicated. Probably the polyploid *Solanum* species would be still more complicated in this respect. In the case of the *S. tuberosum* × *S. curtilobum* hybrids the difficulties are increased by their aneuploid constitution, their male sterility, and the apparent

impossibility of effecting successful back-crossing to the male parent. The features of the assumed cytoplasmic inheritance in these polyploid *Solanum* species are to some extent reminiscent of those found within the genus *Streptocarpus* by OEHLKERS (1940, 1941). However, as little is known about the conditions in *Solanum*, the analogy with *Streptocarpus* may be quite superficial. And lastly it should be pointed out that meiotical aberrations of the same kind as have been found in the male-sterile *S. tuberosum* × *S. curtilobum* hybrids frequently occur in certain varieties of *S. tuberosum*. Such aberrations have been described in almost all the previously cited contributions to the cytology of *S. tuberosum*. This does not imply that the causation is invariably cytoplasmic inheritance or maternal effects, although this is perhaps not excluded in some cases. It would probably be well worth while to make cytogenetical investigations from this point of view on varieties of the species *S. tuberosum*.

## 9. INVESTIGATIONS ON 8x × 4x HYBRIDS.

### A. DESCRIPTION.

Successful crosses between the two tetraploid species *S. acaule* and *S. tuberosum* are rather difficult to make, and are only realized when the former species is used as mother. In the summers of 1941 and 1942 I pollinated altogether 200 flowers of *S. acaule* var. *subexinterruptum* with pollen from *S. tuberosum*, Deodara. The female partner was grafted on tomato. A few parthenocarpic small berries were obtained. The reciprocal cross with grafted plants of Deodara was also attempted on 400 flowers. There, too, a few parthenocarpic berries were obtained but no hybrid seed.

In 1942 ordinary field plants of the artificial octoploid *S. acaule* (cf. p. 96) were pollinated with pollen of Deodara. Out of 25 pollinated flowers one fruit was obtained containing 33 seeds, from which 20 hybrid plants were raised. In the following summer the reciprocal cross was attempted, and 50 flowers of Deodara grafted on tomato were pollinated with pollen from octoploid *S. acaule*. One fruit was obtained, which, however, dropped before full maturity. The fruit contained five seeds, and it remains to be seen whether they germinate, and in case of germination whether they are true hybrids.

The $F_1$ progeny mentioned above showed the following distribution of somatic chromosome numbers:

| 2n | 70 | 71 | 72 | 73 | 74 | Total |
|---|---|---|---|---|---|---|
| No. of plants ..... | 3 | — | 10 | 4 | 1 | 18 |

On two of the twenty hybrids the chromosome numbers could not be determined because of bad fixation. The chromosome numbers of this progeny indicate that numerically unbalanced gametes have functioned. A detailed description of these hybrids will be reserved for a future paper, and only a few preliminary notes will be given here.

The pollen fertility of the numerically balanced hexaploid hybrids ranged from 64 to 83 per cent. On free flowering the seed content of 36 well developed fruits varied between 57 and 113. Some of the numerically unbalanced hybrids were somewhat less fertile. The hybrids were selfed as well as crossed with *S. Rybinii* (2n = 24), *S. tuberosum* (2n = 48) and *S. demissum* (2n = 72).

The hybrid seed of the *S. acaule* (2n=96) × *S. tuberosum* (2n=48) cross germinated quickly in contradistinction to seed of tetraploid and octoploid *S. acaule*. In the early stage of their development the hybrids form a rosette of leaves like that of *S. acaule*, but as development proceeds there grows up from the rosette a stem reaching a height of about 20—30 cm. Like *S. acaule*, the hybrids were characterized by long stolons. Tubers were mainly formed after short-day treatment of the plants. Ordinary cultivated plants only produced very small tubers. The leaves resembled most of all those of the female parent, but like *S. tuberosum* they contained intermittent smaller segments. Anatomically they were characterized by two layers of palisade cells (cf. p. 66). The flowers were light blue, and in size intermediate between those of the parents. They were borne on a branched peduncle, and contrary to *S. acaule* they had articulate pedicels. The hybrids were rather vigorous.

Meiosis was studied in the PMC's of one hexaploid hybrid plant. The average configuration of five cells at diakinesis was $2{,}6_{I} + 29{,}2_{II} + + 1{,}8_{III} + 1{,}4_{IV}$. In fifty II—M plates the number of chromosomes varied between 30 and 38. About 30 per cent of the plates seem to have the balanced chromosome number 36. The sporad stage was very regular, with mainly tetrads.

### B. DISCUSSION.

The tetraploid hybrid between *S. acaule* and *S. tuberosum* has been described by DREMLIUG (1937). Here the pollen fertility ranged from 20 to 30 per cent. On back-crossing this hybrid to *S. tuberosum* DREMLIUG obtained a hexaploid plant, as a consequence of the formation of unreduced gametes in the original hybrid. This plant thus contained 24 *S. acaule* and 48 *S. tuberosum* chromosomes, while the hexaploid

hybrids obtained by me possess 48 *S. acaule* and 24 *S. tuberosum* chromosomes. In the former hybrid the same characteristics also dominated as in my own hybrids.

STELZNER (1943 b) has published the results of similar crosses between *S. acaule* and *S. tuberosum*. Of the former species he used low and high forms. The crosses were most successful when the mother plants were hybrids between high and low *S. acaule*. These *S. acaule* × *S. tuberosum* hybrids were rather fertile. Their somatic chromosome numbers have not been published. Preliminary observations on these hybrids were, however, published by PROPACH (1938 b), who says that usually unreduced egg-cells of the female partner are functioning, so that hexaploid hybrids are obtained. The greater success of the unreduced gametes in these crosses will be discussed later. According to STELZNER (1941), crosses between *S. acaule* and artificial octoploid *S. tuberosum*, variety Pepo, have failed.

## III. DISCUSSION.

### 1. THE RELATIONS BETWEEN THE SPECIES OF THE SECTION TUBERARIUM.

#### A. DIPLOID SPECIES.

The relations between the diploid species of the section *Tuberarium* will now be discussed with reference to the cross *S. Rybinii* × *S. verrucosum* (cf. pp. 22—23). Table 1 shows that most of the subgroups of this section contain diploid species. Diploid inter-specific crosses have mainly been made within and between the closely related sub-groups *Aracciana* and *Andigena* (BUKASOV, 1933, 1938; EMME, 1936 b; KOVALENKO and SIDOROV, 1933; RYBIN, 1933; SCHWARZ, 1937). With few exceptions these crosses have been successful. The $F_1$ hybrids have been fertile and are characterized by a regular bivalent pairing. The unsuccessful cases may at least partly be due to unfavourable climatic conditions (cf. PERLOVA, 1940). Examples are known, however, of intra-group crosses which are difficult to perform and which give feeble non-flowering $F_1$ hybrids, e. g. the cross between *S. Henryi* and *S. chacoense* of the group *Commersoniana* (PROPACH, 1940). This cross was only successful when a certain variety of *S. chacoense* was used.

It may be mentioned that self- and cross-incompatibility have been shown to occur in the two last-named species (STOUT and CLARK, 1924; STELZNER and LEHMANN, 1939). PAL and NATH (1942) have further shown that certain varieties of *S. chacoense* appear to possess, in addition to *S* factors, a fertility factor *F*. DOBZHANSKY (1937, pp. 253—254) gives some examples showing that the viability of

the hybrids in the same two species may depend on the genotype of the particular strains of the parental species used in the cross.

The vigour and fertility of $F_1$ hybrids of inter-group crosses show considerable variation (PROPACH, 1940). The cross between *S. verrucosum* and *S. chacoense* in spite of assumed taxonomical and geographical remoteness resulted in vigorous and fertile hybrids (l. c., p. 124). Inter-group crosses between species of the groups *Bulbocastana, Cardiophylla, Jamesiana* and *Polyadenia* and crosses between species of these and other groups are usually unsuccessful (BUKASOV, 1938, 1940; PROPACH, 1940). In certain cases, e. g. *S. bulbocastanum*, artificial doubling of the chromosome numbers has neutralized this inter-group incompatibility (LIVERMORE and JOHNSTONE, 1940).

If it has been at all possible to obtain flowering hybrids between any two diploid species, meiosis has proved to be fairly regular, even if the species concerned have a widely different geographical origin and are considered by the taxonomists to be very isolated. According to PROPACH (1940), this would prove that the diploid species of the section *Tuberarium* are not structurally differentiated. The evolution of species would here mainly be bound up with gene mutations. Applying the terminology of LEVAN (1937), these species could then be regarded as *autogenomatic*.

Against this hypothesis the following facts may be urged: (1) in the cases of incompatibility mentioned above we do not know whether the chromosome complements are structurally homologous; (2) even if the species are structurally differentiated chromosome pairing may be remarkably regular; (3) since structural heterozygosity, according to the investigations of the present paper, seems to be common within the species, it would be astonishing if the evolution of the species were not at all bound up with chromosomal change in structure; (4) differences between the idiograms of different diploid species have been shown by ŠEPELEVA (1937), e. g. between *S. Henryi* and *S. Vavilovii*. The $F_1$ hybrids between these two species show regular bivalent pairing (PROPACH, 1940). ŠEPELEVA's investigations of chromosome morphology have, however, been discussed earlier in this paper. The cytogenetic studies of the diploid species within the section *Tuberarium* have certainly not advanced far enough to permit a critical judgement of the significance of the facts favouring or rejecting PROPACH's hypothesis. The relationship between autogenomatic species would mainly be expressed by the relative degree of genotypical similarity.

As to the processes which have led to the establishment of distinctions between the species of the section *Tuberarium* we have not far to look for comparisons with the genus *Lycopersicum* (cf. HUMPHREY, 1937) and the *Alata*-group of *Nicotiana* (cf. AVERY, 1938). Comparisons with the pairing behaviour of inter-specific $F_1$ hybrids of these genera have been made on pp. 22—23. LUCKWILL (1939) assumes that the species *Lycopersicum* possesses homologous though not identical sets af factors in their chromosomes. BRIEGER (1930) has shown that in the *Nicotiana alata* group the morphological differences between the species are referable to a few gene differences, but here chromosome re-organization has also operated (AVERY, 1938).

Among non-Solanaceous plants certain genera, e. g. *Antirrhinum* (BAUR, 1932), *Eugalium* (FAGERLIND, 1937) and *Phleum* (NORDENSKIÖLD, 1937), would also probably provide a material well worth comparison with the section *Tuberarium* from the phylogenetical point of view. First of all, however, this section should be more thoroughly investigated.

## B. POLYPLOID SPECIES.

### a. Definitions and introductory notes.

In classifying the polyploid species of the section *Tuberarium* I have followed the definitions of auto- and allo-polyploidy given by MÜNTZING (1936): »The genomes of autopolyploids are structurally identical but may be genetically different. In connection with this structural identity the corresponding (homologous) chromosomes of different genomes have an equal capacity of pairing and crossing-over with each other in any combination. Allopolyploidy, on the contrary, involves structural differences between some of the genomes».

Since these terms have been interpreted in different ways (cf. FAGERLIND, 1937; STEBBINS, 1940), a certain amount of confusion is apt to arise. FAGERLIND has discussed the relations between intra- and inter-specific polyploidy and auto- and allo-polyploidy. According to him, only intra-specific polyploids are absolute autopolyploids. If, however, the original 2x forms have different chiasma frequencies, he reckons with different degrees of absolute autopolyploidy. Among inbred plants of *S. Rybinii* there are probably genotypical differences in chiasma frequencies. Still, it does not seem to me very natural to ascribe different degrees of autopolyploidy to artificial tetraploids of these plants. FAGERLIND points out that autopolyploidy may also be absolute in cases of inter-specific polyploidy. This is in agreement with LEVAN (1937), who states that »on the appearance of amphidiploidy in autogenomatic species autopolyploidy arises (*sensu* MÜNTZING)». On the other hand, allopolyploids derived from hybrids between closely related forms will constitute a transmission to the autopolyploids (DOBZHANSKY, 1937, p. 207).

According to STEBBINS (1940), »the best definition of an autopolyploid is a polyploid of which the corresponding diploid is a fertile species, while an allopolyploid is a polyploid containing the doubled genome of a more or less sterile hybrid». Ideas of this kind have, as we shall see later, been applied by certain authors. The ways of determining whether a polyploid in nature can be cytogenetically assigned to the auto- or allo-polyploids have been discussed by GUSTAFSSON (1943).

### b. Triploid species.

Of the polyploid species, the triploids will first be briefly discussed. Nothing definite is known about their origin. RYBIN (1930, p. 339) thinks that they are probably derived from interspecific 4x × 2x crosses. An intra-specific origin is, however, also possible. In diploid tomato triploid plants now and then spontaneously occur (LESLEY and LESLEY, 1930). At least the two triploid species *S. Commersonii* and *S. chaucha* seem to be decidedly autopolyploid, since their frequencies of trivalents have been even higher than those of the autotriploid tomato.

An attempt to interpret the genotypical relationship between *S. Commersonii* and certain diploid species was made by V. OLÁH (1938), as mentioned earlier. In 3x × 2x crosses the distributions of chromosome numbers found in the $F_1$ progenies differ very much from the binominal distribution that would have been expected if purely statistical factors were determinative. V. OLÁH thinks that there is a skewness in the diploid direction in *aaa* × *aa* crosses, whereas in *aaa* × *bb* crosses the skewness is in the triploid direction (*a* and *b* are here symbols for the genotypes of the haploid chromosome complements). Increasing differences between the genotypes of the 3x and 2x species would thus be reflected in an increased favouring of zygotes approaching the constitution *aab*. Zygotes of the constitution *ab* or approaching this would then of course be non-viable. LEVAN (1942 b) gives a survey of chromosome distributions in 3x × 2x progenies. Somewhat different distributions are also found among different Solanaceous genera, e. g. *Petunia*, *Lycopersicum* and *Datura*, although the crosses recorded according to V. OLÁH are of the *aaa* × *aa* type. In trisomic types of *Nicotiana alata* weak morphological effects from the odd chromosomes are to be found (GOODSPEED and AVERY, 1929 a). In *N. sylvestris* these effects are strong (GOODSPEED and AVERY, 1939). In the light of these facts V. OLÁH's method of studying the relationship between 3x and 2x species does not seem to be so promising.

At any rate, v. OLÁH's suggestions ought to be tested in connection with future investigations concerning the causes of the dissimilar chromosome distributions of different 3x × 2x crosses. Also, 3x × 4x crosses ought to be investigated from this point of view. The possibilities of utilizing synthetized tetraploid plants of the types *aaaa*, *bbbb* and *aabb* in crosses with triploids of the *aaa* type render such investigations still more attractive. If there is any truth in v. OLÁH's hypothesis, it also has a bearing on the problems of incompatibility. Certain species, e. g. *S. bulbocastanum* (2n = 24) and *S. acaule* (2n = 48), that are normally incompatible with *S. tuberosum* (2n = 48) have given viable hybrids with the latter species after the doubling of their chromosome numbers. The hypothetical genotypical constitutions *aa*, *bbcc* and *ddee* of these three species would here explain the success of chromosome doubling with respect to the compatibility. The problems of incompatibility will, however, be discussed later from another point of view.

### c. Tetraploid species.

Among the tetraploid species of the section *Tuberarium*, *S. tuberosum* and *S. acaule* have been the most thoroughly investigated and are therefore of primary concern in the following discussion. These species will be compared with each other and with artificial »true» autotetraploids of *S. Rybinii*.

It is usually assumed that *S. tuberosum* is of hybrid origin (BITTER, 1912, 1913; BUKASOV and JUZEPCZUK, 1929; LONGLEY and CLARK, 1930; ELLISON, 1935; BAYLISS, 1936; IVANOVSKAJA, 1939; MACMILLIAN, 1940), although the opposite opinion has also been expressed (FUKUDA, 1927). Proof of the hybrid origin is considered to lie, among other things, in the occurrence of meiotical aberrations. On page 50 I have shown that these afford no proof of an inter-specific origin. FAGERLIND (1937) has found similar aberrations in assumed intra-specific, autotetraploid types of *Galium verum* and *G. Mollugo*. Whether *S. tuberosum* is of intra- or inter-specific origin is thus still an open question; nor is the origin known of *S. acaule*. Diploid species of the group *Acaulia* have not been found in nature.

GUSTAFSSON (1943, p. 112) points out the importance of the analysis of chromosome morphology when determining the nature of polyploidy. *Solanum*, however, is an unfavourable object for such investigations. We have seen that the diploid species investigated here have two SAT-chromosomes, the artificial autotetraploids four, *S. acaule* four, and *S. tuberosum* two. This last fact speaks in favour of allopolyploidy (MEURMAN and RANCKEN, 1932). Amphiplasty (NAVASHIN, 1928) is, however, not excluded. (In hybrids between certain *Crepis* species it has been found that the satellite contributed by one of the parent species

is permanently lost, whereas in other inter-specific hybrids the satellites from both parents are present.)

On page 52 the existence of a pair of chromosomes corresponding in essentials to the second pair of SAT-chromosomes is suggested. ELLISON (1935) has found differences in the length of the chromosomes within the complements of different varieties of *S. tuberosum*. He is, however, uncertain of the significance of these observations. In his investigations there was some evidence which suggested that in the case of the potato the bud mutation is accompanied by an elongation of the chromosomes in the mutant as compared with those of the parents. This indicates the existence of genotypic control of chromosome length (cf. DARLINGTON, 1937, pp. 53—57).

The results of the analysis of chromosome pairing in *S. tuberosum* have been reported on pp. 52—57. My own investigations indicate a lower frequency of multivalents in two varieties of *S. tuberosum* than in one plant of artificial autotetraploid *S. Rybinii*, but these results should not be generalized. Meiosis has also been studied in polyhaploid ($2n = 24$) *Solanum* plants (LAMM, 1938; IVANOVSKAJA, 1939). In the former case the polyhaploid was of hybrid origin, in the latter case it was derived from pure *S. tuberosum*. Both cases were marked by low pollen fertility in the polyhaploids, and in the majority of the PMC's 12 bivalents were found. The fertility of the corresponding tetraploids was high, and during the first division quadrivalents were observed. In agreeing with the conception of the nature of polyploidy contained in STEBBINS' definition IVANOVSKAJA has taken these facts as evidence of the allopolyploid nature of *S. tuberosum*. To me it rather seems that the combined occurrence of regular pairing in the 2x form and of multivalents in the 4x form indicates a mainly autopolyploid constitution of the latter. The bivalent pairing of the 2x form may partly be due to differential affinity (a phenomenon that will be discussed below).

Autotetraploid tetrasomic inheritance in *Solanum tuberosum* was first shown by MÜLLER (1930), and later by LUNDEN (1937), who studied the inheritance of twelve different factors. The complementary factor *D* for colour showed random chromatid segregation. According to SANSOME (1933), there must be an approximately 100 per cent quadrivalent formation to obtain random chromatid segregation. The four chromosomes containing the *D* loci would thus very frequently be united in a quadrivalent. LUNDEN (1937) suggests that the chromosomes appear as quadrivalents at diplotene but later at diakinesis and I—M

become separated into bivalents. This hypothesis, however, seems rather dubious (cf. UPCOTT, 1935).

The theories of segregation in autotetraploids have been further worked out by MATHER (1935 b, 1936 b). Ordinary autotetraploid segregation can, of course, also occur in an autotetraploid where the potential quadrivalents are realized as bivalents only. On page 56 I have shown that the ring-quadrivalents in *Solanum* almost always occur in the non-zigzag form. Now if such a ring-quadrivalent is formed by chromosomes each having one long and one short arm, it seems to me quite possible that the long arm will usually be orientated parallel to the spindle whereas the short one will be parallel to the equator (see Fig. 118). Double reduction will then frequently occur of the distal ends of the short arms. If the loci for *D* should happen to be situated distally in the short arms of these chromosomes, for instance, in the offspring of triplex *DDDd* forms, nulliplex *dddd* plants would be comparatively common. Approximately random chromatid ratios could then perhaps be obtained with much less than 100 per cent quadrivalent formation. There is, however, no proof of the correctness of these suggestions.

It seems to me that the polyploidy in *S. tuberosum* is mainly autopolyploidy with slight differentiations in the allopolyploid direction. The nature of the polyploidy in *S. acaule* is more difficult to judge, above all because of the lack of genetical investigations.

In *S. acaule* the occurrence of four SAT-chromosomes speaks in favour of autopolyploidy, but the regular bivalent pairing indicates allopolyploidy. In the triploid $F_1$ hybrid *S. acaule* × *S. chacoense* (PROPACH, 1937 b) there is a comparatively high frequency of trivalents. For this reason PROPACH has drawn the conclusion that *S. acaule* is actually autotetraploid. He writes (l. c., p. 151): »Die Allopolyploidie der Arten *S. acaule* und *S. demissum* ist nur eine scheinbare, da in ihren Bastarden mit diploiden Analysatoren die Paarung genau so verläuft wie bei einem Autopolyploiden. Ihre Genome müssen also homolog sein, was zytologisch in der strengen Bivalentenpaarung der reinen Arten nicht zum Ausdruck kommt». Similar cases have been described by MÜNTZING (1936) as well as MÜNTZING and PRAKKEN (1940). According to their opinion the two-by-two pairing of these polyploids »can only be explained by assuming a special genotypically controlled tendency to bivalent formation. In species capable of such a control the formation of multivalents is prevented, and thus a regular meiosis is secured in spite of complete or almost complete homology between more than two genomes. Only when there is no opportunity for pairing two-by-two are multivalents formed».

The meiotic behaviour of the artificial octoploid *S. acaule*, however, could not be explained by their hypothesis, since the mode of chro-

mosome pairing here was the same as in autotetraploid *S. Rybinii*. Multivalents of higher order than quadrivalents were not observed. If the bivalent pairing of tetraploid *S. acaule* really was genotypically controlled, this control on doubling the chromosome number by colchicine treatment would either be maintained or be destroyed. In the former case the octoploid form ought also to show two-by-two pairing, in the latter case it seems rather strange that higher associations than quadrivalents do not occur. If, however, *S. acaule* is an allotetraploid species, the meiotic behaviour of the 4x and 8x types is in agreement with expectation. FAGERLIND (1937) is of opinion that he has found correlation

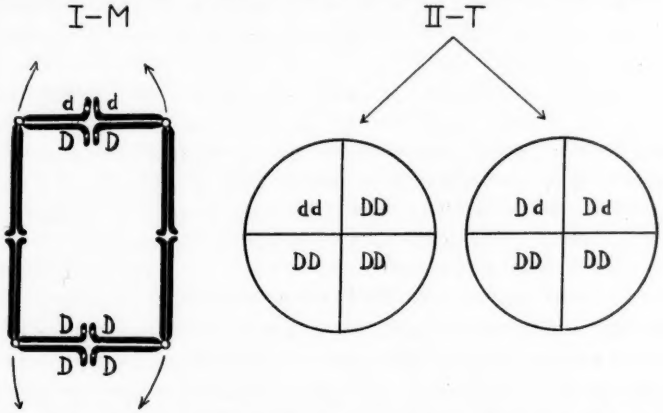



Fig. 118. — Hypothetical quadrivalent arrangement and co-orientation in a plant triplex for »D». If the quadrivalent was always non-zigzagly arranged and co-orientated with the long arms parallel to the spindle, the resulting gametic series would be 5DD : 2Dd : 1dd. Random arrangement and co-orientation would give the series 15DD : 12Dd : 1dd.

between chromosome-diminution and the type of pairing in *Galium*. With a corresponding degree of autopolyploidy species having large chromosomes are characterized by multivalent pairing, species with small chromosomes by two-by-two pairing. The chiasma frequency should be lower in the species last mentioned. The chromosomes of *S. acaule* are certainly smaller than those of *S. tuberosum* and *S. Rybinii*, but the chiasma frequency is probably by no means lower.

The occurrence of trivalents in the $F_1$ hybrid *S. acaule* × *S. chacoense* may be due to differential affinity (cf. DARLINGTON, 1937, pp. 201—202). On the other hand, in hexaploid *S. acaule* plants, which I intend trying to obtain from intra-specific 8x × 4x crosses, the differential

affinity would probably contribute to a decrease of the expected frequency of trivalents in favour of a two-by-two pairing. Since forms that differ in plant height, division of leaf and colour of fruit are known in *S. acaule*, genetical investigations could probably be made. These would most likely afford the best criterion of the nature of the polyploidy. And lastly it may be pointed out that the pollen grains of *S. acaule* have three germination pores, whereas grains with four pores are common in the pollen of *S. tuberosum* and artificial autotetraploids. I have earlier mentioned that the comparatively small cell-size of *S. acaule* cannot be used as evidence of allopolyploidy. I have also stated that *S. tuberosum* seems to suffer much more from the doubling of the chromosomes than *S. acaule*, and this holds good for both plant vigour and fertility.

Most of the circumstances mentioned indicate that *S. acaule*, at least relatively to *S. tuberosum*, has the nature of an allopolyploid species. STEBBINS (1940) points out that the most complex, but probably also the most common situation in plants, is that in which both auto- and allo-polyploid types exist together in the same complex of related species. Here the section *Tuberarium* will serve as a good example.

### C. SECONDARY POLYPLOIDY.

Another complication must be added to that presented by the conditions of polyploidy in *Solanum*, viz. the hypothesis that the true basic number of the Solanaceous polyploid series is 6 and not 12. This hypothesis has been discussed in the Chapter on secondary association. It has been assumed that secondary polyploidy should reveal itself in the occurrence of (1) secondary association, (2) secondary balance, (3) autosyndesis within the haploid chromosome set (in *Solanum* consisting of 12 chromosomes), (4) chromosomes with similar chromosome morphology within the haploid set, and (5) in complex hereditary behaviour. The results obtained from studying secondary association within the section *Tuberarium* were rather heterogeneous, and it was impossible to point to any definite chromosome number less than 12 as being the basic one. Neither was a secondary balance in favour of the assumed basic chromosome number 6 found in the $F_1$ progenies of certain 3x $\times$ 2x and 3x $\times$ 4x crosses. WANSCHER (1934) assumes the basic chromosome number of the Solanaceous plants to be 4, but this hypothesis would also be impossible to prove on the basis of the results of the present investigation. Some authors believe they have found a high degree of autosyndetic pairing in the triploid *Solanum*

species and hybrids, as was mentioned in the Chapter on a triploid species. The investigations on which this belief rests were probably not very reliable, however, and fresh ones ought to be undertaken.

Haploid plants of the diploid species of the section *Tuberarium* have so far not been found, but some haploids with 12 chromosomes of other related genera have been investigated. In haploid *Lycopersicum esculentum* (LINDSTROM and KOOS, 1931), *Datura stramonium* (BELLING and BLAKESLEE, 1922) and *Nicotiana glutinosa* (GOODSPEED and AVERY, 1929 b; WEBBER, 1933) only univalents were observed at the first division, while KOSTOFF (1941) considers that he has found from 0 to 5 bivalents in haploid *Nicotiana sylvestris*. With regard to the morphology of the chromosomes of *N. sylvestris* KOSTOFF (1941) declares that ten of the chromosomes can be classified into five similar pairs, while two of them are dissimilar. On the strength of these observations he states among other things that the basic chromosome number in *Nicotiana* is obviously 6. The morphology of the chromosomes in *N. sylvestris* has also been described by GOODSPEED and AVERY (1939). This description does not agree with KOSTOFF's. There are, however, considerable variations in the chromosome morphology of different *Nicotiana* species (also between the 12-paired), and GOODSPEED (1934) states »that the caryotype of the *Nicotiana* species furnishes little evidence in respect to their possible deviation from one another or from common ancestral types». The differences in the chromosome morphology of *Solanum* and *Lycopersicum* have already been pointed out. To this group, which on comparison shows considerable differences, *Datura* (BELLING and BLAKESLEE, 1924; BERGNER, 1939) could be added. The method of inferring supposed original basic chromosome numbers from the chromosome morphology has been criticized by LEVAN (1942 a, pp. 207—208), and I think that the examples given above also show the difficulties of applying this method to the Solanaceous plants. And, lastly, the genetical method calls for some comment.

In *Pyrus* genetic evidence indicates more complex conditions than those found in strictly diploid organisms (DARLINGTON, 1937, p. 240). Genetical investigations on diploid *Solanum* species of the section *Tuberarium* have been made by EMME (1936 b) and PROPACH (1940). PROPACH erroneously believes that 35 : 1 segregations would occur if the original basic chromosome number was 6, while in reality 15 : 1 ratios should be expected. Since only monofactorial ratios were obtained, he concludes that the basic chromosome number is 12. Too few genes have, however, been investigated to justify such a conclusion. In the

closely related genus *Lycopersicum* more exhaustive genetical studies have been made (MACARTHUR, 1934; BOSWELL, 1937). Also here monofactorial ratios have been obtained. Two 15 : 1 segregating instances are known, however (SCHLÖSSER, 1936; LESLEY and LESLEY, 1939). In *Dahlia* LAWRENCE (1931 c) suggests that certain pairs of dissimilar genes are of common origin and have been differentiated later. In analogy with this highly hypothetical suggestion it could be assumed that the factors $A_1$ and $A_2$, $D_1$ and $D_2$, $R$ and $T$, of the tomato (cf. BOSWELL, 1937) are »polymeric». It should also be remembered that the 15 : 1 segregation alone furnishes no critical proof that duplicate chromosomes are involved (cf. EYSTER, 1934). HEILBORN (1935, p. 174), arguing from the unexpected behaviour shown by the pollen fertility of certain interchange heterozygotes in *Datura stramonium*, has suggested that this species is perhaps no real diploid but a secondarily balanced type with some of the chromosome parts duplicated.

All these facts show that no proof is as yet forthcoming that the original basic number of the Solanaceous plants was 6. On the other hand, it would be too hasty an inference to assume 12 as the original basic number. This number is rather high as compared with the basic chromosome numbers of certain genera belonging to the family Solanaceae and to related families (cf. KOSTOFF, 1941—1943, pp. 55 and 1012). It is, however, impossible at present to point out a definite chromosome number less than 12 as the basic one.

### D. INCOMPATIBILITY.

A phenomenon which should be considered in connection with the relations between the species is incompatibility — failure or difficulty of crossing. The degree of incompatibility has been taken as evidence of more or less remote relationship between certain *Solanum* species (SIDOROV, 1937; BUKASOV, 1938), whereas PROPACH (1938 b) states that there is no correlation between incompatibility and relationship. In this connection it seems probable, however, that the following conclusions of WATKINS (1935, p. 200) are also applicable to the section *Tuberarium*: »Altogether, it is clear that success or failure in crossing two species depends on the interplay of various independent causes. It is easy to see how it happens that, although it may be impossible to cross two nearly related varieties and possible to cross species from different genera, yet it remains true on the whole that the more distant the relationship the less the chance of a successful cross».

In the present investigation »one-way» incompatibility occurred

in certain crosses between *S. chaucha*, *S. acaule* and *S. curtilobum*. In the last case, however, this incompatibility was not absolute. It has also been mentioned that certain crosses between diploid species are not successful. »One-way» incompatibility between species with different chromosome numbers is rather common, e. g. between 4x and 2x species, and here sometimes the 4x × 2x combination is compatible, sometimes the reverse (cf. STELZNER and LEHMANN, 1939, p. 107). »One-way» incompatibility may even occur between closely related diploid species of the same group, e. g. between *S. aracc-papa* and *S. Bukasovii* (EMME, 1936 b). Certain species are almost always easy to cross in both directions with other related species. Within the group *Tuberosa* this seems to be the case with *S. Rybinii*, while other species, e. g. *S. ajanhuiri*, are usually only successful as female partners (cf. KOVALENKO and SIDOROV, 1933; EMME, 1936 b). Sometimes the incompatibility can be neutralized by the doubling of the chromosome number of one of the partners. It has also been found that unreduced gametes of one of the parents are sometimes regularly functioning, e. g. in the cross between *S. tuberosum* and *S. Antipoviczii* (IVANOV, 1939). Similar conditions of incompatibility are also found in other genera, e. g in *Nicotiana* (KOSTOFF, 1930 b) and in *Galium* (FAGERLIND, 1937).

Incompatibility is mainly caused by arrested pollen tube growth or by zygotic lethality. Its causes have been exhaustively discussed by MÜNTZING (1936, pp. 326—334) and FAGERLIND (1937, pp. 433—444). In the section *Tuberarium* the causes of incompatibility have been investigated in only a very few cases. The conditions among the diploid species of this section ought to be studied first. I shall here only point out a few facts that might be of interest in reference to the problems of incompatibility within the section *Tuberarium*.

According to PROPACH (1940), all diploid species of the group *Stellata* are self-sterile. Among the diploid *Rotata* we have seen that *S. verrucosum* is self-fertile. *S. Rybinii* and *S. stenotomum* are allogamous, it is true, but they also give seed after selfing. Self- and cross-incompatibility in accordance with the oppositional factor hypothesis (cf. SANSOME and PHILP, 1932) has been shown to occur among some species of the group *Stellata* and in *S. aracc-papa* of the group *Rotata*. (STOUT and CLARK, 1924; PAL and NATH, 1942). Hitherto, however, comparatively few species have been investigated. The self- and cross-incompatibility of autotetraploid *Solanum* has been discussed by LEWIS (1943), whose ingenious work on induced autotetraploids of *Oenothera organensis* contributes very much to the knowledge of incompatibility in

polyploids. v. KESSELER (1930) has shown that in *S. tuberosum* one and the same variety may provide successful male partners in some crosses but may fail to do so in others. Examples of »one-way» incompatibility between polyploids have been given in the present work. On the basis of the oppositional factor hypothesis cases of this kind should be expected among the polyploids (LAWRENCE, 1930; LEWIS, 1943). No doubt this hypothesis would also explain some of the cases of »one-way» incompatibility between diploids (cf. SANSOME and PHILP, 1932, pp. 22—24) and the success of certain crosses after the doubling of the chromosome number. Other conditions must also, however, be taken into consideration.

The bearing of v. OLÁH's hypothesis on incompatibility has already been discussed. In crosses of plants having different chromosome numbers the chromosome number relation *mother plant* : *embryo* : *endosperm* is certainly of importance (cf. MÜNTZING, 1933 b). The crux of these questions is, however, the causes of incompatibility and »one-way» incompatibility between diploid species. Here, several hypothetical explanations could be given. FAGERLIND (1937) points out the importance of the osmotic conditions, KOSTOFF (1930 b) considers that failure of seeding is due to an immunity reaction between the genotypically diverse endosperm and the adjacent maternal tissues. These last assumptions have, however, been criticized by COOPER and BRINK (1940, pp. 614—615). In this paper I especially want to point out the probable importance of cytoplasmic conditions. Elimination of zygotes or gametes of definite genotypical constitution under the influence of the plasm may sometimes occur (cf. EAST, 1932; SIRKS, 1938). The favourable influence of chromosome doubling on the compatibility might sometimes also be explained from this point of view. KIRSCH (1941) has shown that the balance between the haploid genome of *Epilobium hirsutum* and the cytoplasm of *E. luteum* is less favourable than the balance between the same cytoplasm and the diploid genome of the former species. Until the causes of the incompatibility between the diploid *Solanum* species have been thoroughly studied I think it will be rather difficult to interpret the conditions of incompatibility among the polyploid species of which from the practical point of view *S. tuberosum* is the most important object for such investigations. The causes of zygotic lethality will be discussed below in connection with the occurrence of diplontic and somatoplastic sterility.

## 2. STERILITY.

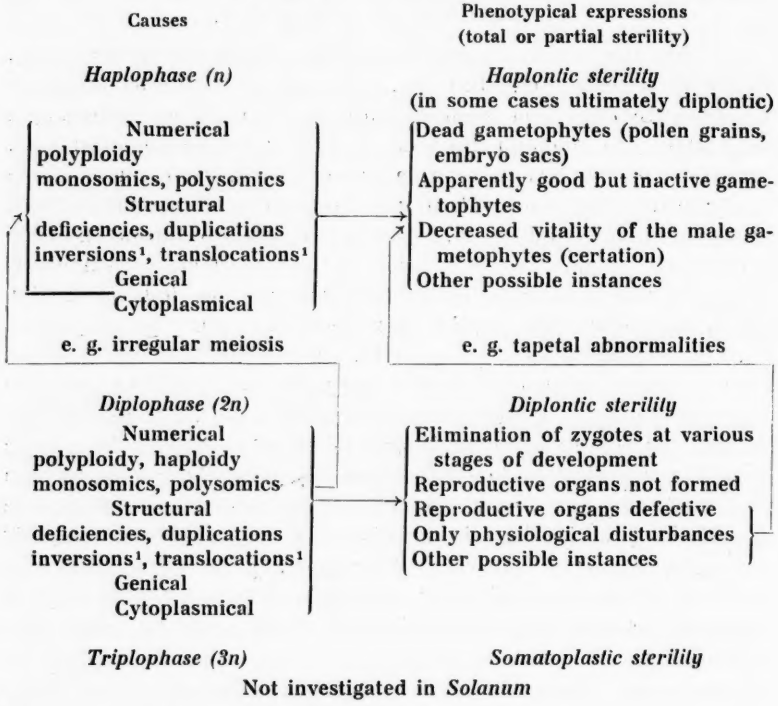
### A. CLASSIFICATION OF STERILITY.

Within the section *Tuberarium* various kinds of sterility occur. *Modificatory sterility* (cf. MÜNTZING, 1930) caused by external influences of various kinds has been discussed earlier and will only be touched upon incidentally. In *Solanum* this kind of sterility has an important rôle. *Correlative sterility*, which has been discussed on p. 10, should of course be assigned to this class. *Sterility in the strict sense* is characterized, according to MÜNTZING (1930), by lacking or defective formation of the reproductive organs or their products under »normal» conditions. MÜNTZING further distinguishes between *haplontic* and *diplontic sterility*, and in his review of the sterility phenomena (l. c., pp. 314—323) shows what these conceptions should signify. Following MÜNTZING's ideas I have proposed a scheme of sterility types and their assumed causes in *Solanum* (Table 21). A third category of sterility, so-called *somatoplastic sterility*, has also been included. For this kind of sterility the scheme has not been worked out in detail, somatoplastic sterility not having been studied in *Solanum*. The scheme submitted is mainly intended to serve as a starting-point for the following discussion and lays no claim to being a complete survey of the sterility phenomena among higher plants. A complete scheme accounting for maternal effects, interaction between somatoplastic and diplontic sterility and so on would be much more complicated.

The arrows in Table 21 between the haplophase and diplophase indicate that some cases of haplontic sterility may ultimately be diplontic (cf. MÜNTZING, 1930). It might also be questioned whether from the point of view of the zygote certain cases of diplontic sterility should not ultimately be classified as modificatory. In crosses of *Linum* and *Galeopsis* (l. c., p. 318) the diplontic sterility was not inherent in the zygote itself. Here the death of the zygotes was occasioned by the environments provided by the female parents. It seems to me that perhaps these parents do not represent the »normal» conditions for the further development of the hybrid embryos. In these cases I would sooner assign to the expected hybrid plants the »normal» environmental conditions for the development of these hybrid zygotes. This conception seems in any case to be reasonable if there are great differences between the parent plants. The causes of sterility listed in Table 21 may in turn be caused by inbreeding, intra- and inter-specific hybridization and mutation (*sensu* STURTEVANT and BEADLE, 1939, p. 206).

The types of diplontic sterility characterized by an elimination of zygotes at an early stage of development are of special interest for the problems of incompatibility. The significance of genical, numerical, physiological and cytoplasmical conditions has been touched upon in

TABLE 21. *Sterility types and their assumed causes in Solanum.*




the discussion of incompatibility. In this connection the probable importance of somatoplastic sterility should also be mentioned. Especially the inter-specific *Solanum* crosses in which the incompatibility changed into compatibility after the doubling of the chromosome number of one of the partners should afford interesting material in which to study this kind of sterility before and after the chromosome doubling.

Thanks to the work of COOPER and BRINK (1940) and BRINK and COOPER (1940, 1941) complete somatoplastic sterility has been shown in the cross *Nicotiana rustica* ($2n = 48$) $\times$ *N. glutinosa* ($2n = 24$) and certain other incompatible crosses, whereas incomplete somatoplastic sterility occurred in the cross *Nicotiana rustica* ($2n = 48$) $\times$ *N. tabacum* ($2n = 48$) and after self-pollination of *Medicago sativa*. In the first cross all the fertile ovules, in the second a great proportion of them, collapsed at an early stage of development. This collapse was the result of retarded endosperm

[1] Presumably inversions and translocations *per se* only cause haplontic and diplontic sterility to the extent they may give rise to positional effects.

growth, pronounced hypertrophy of the nucellus and integument, and a disorganization of the vascular bundles nourishing the embryo and endosperm. It was presumably caused in the inbred *Medicago sativa* by the bringing together in homozygous form of certain unfavourable recessive genes, and in the hybrid *Nicotiana* seed by the inharmonious reaction of widely different genomes. Somatoplastic sterility was assumed to be influenced by the maternal genotype as well as by the genotype of the endosperm, and hence both diplophase and triplophase differences were probably involved.

B. THE STERILITY PHENOMENA OF THE SECTION TUBERARIUM.

In the diploid allogamous species *S. stenotomum* and *S. Rybinii* there seems to be a causal connection between pollen sterility (total and partial) and structural hybridity. The plants investigated were slightly inbred. It would be of great interest to study the frequency of structural hybridity among population plants of these species. Haplontic sterility caused by numerical or structural aberrations is probably rather common among allogamous diploid species (cf. MÜNTZING, 1939). The sterility of these diploid *Solanum* plants may also to some extent be diplontic, as is probably the case in rye (cf. MÜNTZING and PRAKKEN, 1941), and certainly modificatory sterility due to unfavourable climatic conditions also plays an important part.

The effect on sterility of gain or loss of chromosomes in relation to both the diploid and the tetraploid number has been studied in the progenies of certain inter-specific crosses. In the former case, however, only the effect of gain was studied. In *Lycopersicum*, LESLEY and LESLEY (1941) found a diploid plant heterozygous for a deficiency. This plant produced seeds after selfing. Some polysomic *Solanum* plants were obtained from the 3x $\times$ 2x cross between *S. chaucha* and *S. stenotomum*. Here both diplontic and haplontic sterility occurred. Plants with more than one extra chromosome did not flower, but for one exceptional plant of the constitution 2x + 1 + 1. Double trisomic plants of this constitution are usually more vigorous than those of the 2x + 2 constitution (SANSOME and PHILP, 1932, p. 254). As might be expected, there was a considerable variation of the pollen fertility between the trisomic plants. The triploid *S. chaucha* was completely male-sterile, although the pollen contained about 20 per cent apparently good grains. This is probably partly due to diplontic sterility, partly to modificatory (cf. p. 35). In the 2x $\times$ 3x tomato cross carried out by LESLEY (1928) only two plants were obtained, both of which were diploid. In some of the trisomic types obtained from the reciprocal cross, however, certain extra chromosomes were transmitted through the pollen. This indicates

that triploid tomato is also characterized by diplontic sterility. Among the lower chromosome numbers between 2x and 3x the balance of the genotype is more easily upset than among the higher chromosome numbers. Among the hybrids of the 3x × 4x cross between *S. chaucha* and *S. tuberosum* the average fertility increases with increasing chromosome number. Single plants with a comparatively high percentage of apparently good pollen even occurred among the 4x—6 plants, and some plants with 4x—3 chromosomes were spontaneous seed setters.

Plants with more than 48 chromosomes were obtained in crosses with the pentaploid *S. curtilobum* and its derivatives. In the 5x × 4x crosses *S. curtilobum* × *S. andigenum* and *S. curtilobum* × *S. tuberosum* and in the 4x × 5x cross *S. tuberosum* × *S. curtilobum* hybrids with chromosome numbers ranging from 48 to 59 occurred. Hybrids with intermediate chromosome numbers were the most common. Here the chromosomes in excess of 48 had, on an average, no significant influence on pollen fertility or plant vigour, i. e. there was no correlation between chromosome number and these characteristics. In *Dactylis*, MÜNTZING (1937) has found a similar situation. In the back-cross of a 4x + 4 $F_1$ hybrid of the cross *S. tuberosum* × *S. curtilobum* to its female parent (cf. p. 85) there was a deviation in the distribution of chromosome numbers in the tetraploid direction. A similar but still stronger deviation was found in the selfed progeny of *S. curtilobum* (cf. p. 65). In *S. tuberosum* unbalanced gametes are obviously formed, yet plants with deviating chromosome numbers do not occur, as is, for instance, the case in the tetraploid *Dactylis glomerata* (l. c., p. 163) or in artificial autotetraploid *Galeopsis pubescens* (MÜNTZING, 1941). It seems not unlikely that these different *Solanum* cases could be explained by differences in somatoplastic sterility. When crossing or selfing *S. tuberosum* and when selfing *S. curtilobum* the genotypic similarity between mother plant and endosperm is probably greater than in the crosses between these species. In the latter cases hybrid vigour of the endosperm can perhaps nullify the somatoplastic selection in favour of numerically balanced hybrid seed. The behaviour of the back-cross progeny is also in agreement with this hypothesis. Besides this, competition between balanced and unbalanced gametes may be of importance. The proportion of numerically balanced gametes is certainly much higher in *S. tuberosum* than in *S. curtilobum*. LESLEY (1928, p. 34) has shown that in tomato the hybrid triplo-C parent transmits the extra chromosome somewhat more readily than the non-hybrid. He assumes that »this may be due in part to the superior germination of the hybrid seed compared with the non-hybrid». The

collapse of fertilized ovules in *S. tuberosum* has been observed by CLARKE (1940).

As mentioned earlier, female-fertile octoploids of *S. tuberosum* have been artificially produced. By repeated back-crossings it would therefore probably be possible to obtain artificial pentaploid *S. tuberosum*, and an intra-specific 5x × 4x crossing could be carried out. By this method and probably also by others my hypothesis concerning the absence of hypo- and hyper-tetraploids in *S. tuberosum* could be experimentally tested.

As regards pollen fertility, chromosome doubling in *S. Rybinii* has given very different results. Various *Solanum* species also seem to react differently (cf. pp. 40—42). In *Galeopsis pubescens* chromosome doubling may give different results, depending upon the genotype of the original diploid line (MÜNTZING, 1941). Apart from these differences the doubling of the chromosome number of *Solanum* species is followed by a general decrease in fertility. DARLINGTON (1937, p. 196) ascribes the reduced fertility of autotetraploids to the unequal segregation of multivalents. UPCOTT (1939) points out that fertility in autotetraploids could be obtained either by low chiasma frequency or by a regular formation of quadrivalents of a certain type. MÜNTZING (1936, pp. 317—320) suggests that the reduced fertility of experimental polyploids is caused to a great extent by physiological disturbances and is thus diplontic. In his paper on *Dactylis* (MÜNTZING, 1937, p. 223) he further points out »that sterility in aneuploids (and autopolyploids) may also be diplontic and not only haplontic». FAGERLIND (1937, p. 419) has given a comparative scheme of the fertility and sterility of 2x and 4x types.

Since chromosomal aberrations were frequency found among plants of the diploid *Solanum* species, the question arises whether similar aberrations are not to a great extent responsible for the reduced fertility of the polyploids. In polyploids aberrations of this kind probably have a less deleterious effect and may consequently accumulate to a considerable degree (cf. HEILBORN, 1935; HUSKINS, 1941). The former author (l. c., p. 173) thought that partial pollen sterility in polyploid plants may be due to structural heterozygosity with respect to deficiencies, while MÜNTZING (1938, p. 180) points out that in polyploid species it is less probable that any structural changes occurring will result in visible pollen abortion. This latter suggestion probably refers to the quantitatively more or less insignificant structural aberrations. If, for example, a diploid plant of *S. Rybinii* is heterozygous for a deficiency, the corresponding autotetraploid plant will be duplex for the same aberration. Hence one-sixth of the gametes will be aborted if the

deficiency is lethal in homozygous condition. Even if several structural differences are involved, the occurrence of gametes homozygous for one or more of these changes will be more infrequent than in the corresponding diploids. On the other hand, as the polyploids probably allow of a greater accumulation of structural aberrations, it is difficult to say whether such aberrations are of less importance for haplontic sterility in the polyploids than in the diploids. ELLISON (1935) thinks that in *S. tuberosum* structural hybridity is one of the causes of the sterility, but the uncertainty of his investigations has been pointed out earlier.

Genes causing haplontic sterility may occur in *Solanum*. KRANTZ, BECKER and FINEMAN (1939) have studied the inheritance of pollen sterility in *Solanum tuberosum*. They assume tetrasomic inheritance for a gene designated *V*. All the *vv* pollen and about 80 per cent of the *Vv* pollen aborted. MÜNTZING (1938, p. 183) points out that some self-sterility alleles in a homozygous condition have a more or less lethal effect. In some polyploid *Solanum* species the haplontic sterility of certain gametophytes may be caused by the homozygosity of such genes.

A gene-determined diplontic sterility is, however, probably of still greater importance. Here, more especially the conditions within *S. tuberosum* will be discussed. Of this species, varieties are known characterized by restricted habit of bloom, rudimentary to weak inflorescence, ready abscission of flower and poor development of stamens (KRANTZ, BECKER and FINEMAN, 1939). Irregular meiosis, mainly in the PMC's of this species, has been described by FUKUDA (1927), STOW (1927), HEYN (1930), LONGLEY and CLARK (1930), BLEIER (1931 a), MEURMAN and RANCKEN (1932), BAYLISS (1936), and ELLISON (1936 a, 1937). Similar gene-controlled meiotical irregularities are found in other Solanaceous plants. In *Lycopersicum esculentum*, for example, a timing factor is probably responsible for certain meiotical irregularities (LAMM, 1944 b), and the asynaptic behaviour of a *S. Rybinii* plant described in the present paper may be gene-controlled, just as is asynapsis in *Datura* (BERGNER, CARTLEDGE and BLAKESLEE, 1934) and numerous other plants. In *S. tuberosum* no direct correlation can be established between the sterility of the male gametophyte and that of the female (BAYLISS, 1936). Very few varieties are completely female-sterile, whereas total male sterility frequently occurs. This also indicates the predominant importance of diplontic sterility. Here I would call to mind the importance of tapetal degeneration and of the cytoplasmic conditions earlier discussed. Of considerable interest in this connection are the investigations of GENTCHEFF and GUSTAFSSON (1940) and

GENTCHEFF (1941) concerning the influence of the tapetum on meiosis. That irregular meiosis may not be due to a hybrid origin of *S. tuberosum* has been pointed out earlier, nor may this be the case with the cytoplasmic differences probably existing within this species (cf. SALAMAN and LESLEY, 1922). In *Epilobium parviflorum* plasmic differences exist between biotypes within the species (cf. SIRKS, 1938), and according to MICHAELIS (1943), within *E. hirsutum*, the very deviating cytoplasm of the biotype »Jena» is probably of intra-specific origin. By these statements I do not mean to say that *S. tuberosum* cannot be of interspecific origin.

The existing varieties of *S. tuberosum* probably originate from the selfed progenies of a few plants that were introduced early into Europe (cf. p. 6) and from crosses between them (Imperial Bureau, 1936). One would therefore be inclined to surmise a general inbreeding of this species, which would partly explain the frequent occurrence of sterility. As against this conjecture, however, the varieties of *S. tuberosum* seem to be rather heterozygous and if inbred degenerate (cf. STEVENSON and CLARK, 1937; STELZNER and LEHMANN, 1939). The object of practical plant breeding has not at all been to improve the fertility of *S. tuberosum*. Hence types of unsatisfactory fertility have not been removed by selection. The purpose of the present investigation, however, has not been to make a special study of the causes of the sterility found among the varieties of *S. tuberosum*, though I think that some of the results obtained may none the less be of interest in this connection. With respect to the general problems of fertility and sterility within the section *Tuberarium* it may be said that more thorough investigations of the diploid species and of artificial polyploids are desirable.

## SUMMARY.

(1) The purpose of the present investigation is to elucidate the conditions of primary and secondary polyploidy, incompatibility and sterility within the section *Tuberarium* of the genus *Solanum*. This has been attempted mainly by cytological investigations of pure species, artificial polyploids and hybrids.

(2) Investigations of the morphology of the somatic chromosomes show that the chromosome complement of the diploid species *S. Rybinii*, *S. stenotomum* and *S. verrucosum* and the tetraploid species *S. tuberosum* contains two SAT-chromosomes, whereas the number of SAT-chromosomes is four in artificial autotetraploids of *S. Rybinii* and in the natural

tetraploid species *S. acaule* var. *subexinterruptum* (abbreviated in this paper to *S. acaule*). The latter species has comparatively small chromosomes. Besides SAT-chromosomes, some secondarily constricted chromosomes can be distinguished from the rest of the complement. Examinations of the interphase of meiosis show that occasionally other chromosomes than the SAT-chromosomes may function as nucleolus-organizers.

(3) In the slides of chilled root-tips the chromosomes were super-contracted. The contracted, almost spherical, somatic chromosomes of *S. acaule* show secondary association. The cold treatment revealed a probable heterochromatic constitution of some chromosome ends.

(4) In slightly inbred progenies of the allogamous diploid species *S. Rybinii* and *S. stenotomum* there was a considerable inter-plant variation of pollen fertility. Meiosis was studied in some of these plants representing various degrees of fertility. Two male-sterile plants as well as most of the plants having a high degree of partial pollen sterility were characterized by chromosomal aberrations, e. g. inversions and translocations. A reciprocal translocation was also found in a partially sterile plant of the autogamous diploid species *S. verrucosum*. In the diploid *Solanum* species there seems to be a causal connection between sterility and chromosomal aberrations.

(5) The general meiotic behaviour of some non-aberrant diploid plants was studied. The chiasma frequency (mean number of Xta per bivalent) varied between 1,26 and 1,68, the terminalization coefficient between 0,78 and 0,92. The meiotic behaviour of two $F_1$ hybrids between *S. Rybinii* and *S. verrucosum* was fairly normal, and did not indicate the existence of coarse structural differences between these species. Negative correlation of chiasma frequencies was also studied. The results obtained were, however, too divergent to permit conclusions in the one or the other direction.

(6) Meiosis of the diploid plants showing chromosomal aberrations is described. Among these plants a diploid-tetraploid chimaera of asynaptic *S. Rybinii* was the most interesting. This chimaera was obtained after colchicine treatment of a diploid seed. In several respects these asynaptic plants were reminiscent of »sticky» maize. The stickiness of the chromosomes during the first and second division of meiosis may be due to nucleic-acid starvation.

(7) In the triploid species *S. chaucha* the frequency of trivalents was rather high. A slight degree of autosyndesis was observed in a few PMC's, but these observations may be faulty. At I—A divided uni-

valents were occasionally observed. The occasional division of univalents at the first division of meiosis has also been observed in some aneuploid *Solanum* hybrids.

(8) When *S. chaucha* was used as female parent in crossings with *S. stenotomum* and *S. tuberosum* it was grafted on tomato. This procedure was also applied in other inter-specific crossings. The pollen fertility of the highly fertile autogamous tetraploid species *S. acaule* was unaffected by grafting, whereas the pollen fertility of the variety Deodara of *S. tuberosum* was decreased as a consequence of grafting.

(9) The effect of doubling the chromosome number on fertility has been studied mainly in the diploid species *S. Rybinii* and in the tetraploid species *S. acaule*. As regards fertility, chromosome doubling gave very different results, depending upon the genotype of the diploid colchicine-treated seed. Artificial octoploids of *S. acaule* had a remarkably high fertility. Other authors have shown that artificial octoploids of *S. tuberosum* either do not flower, or if they flower have a very low fertility.

(10) The results of cytological investigations on artificial tetraploids of *S. Rybinii*, natural tetraploids of *S. tuberosum*, *S. andigenum* and *S. acaule* and artificial octoploids of *S. acaule* have been summed up on page 58, and need not be repeated here.

(11) The effect of chromosome doubling on pollen and stoma size and on certain morphological characteristics has been studied. In *S. Rybinii* the heterozygosity among the diploids was reflected by a considerable variation among the artificial tetraploids. Here, for example, diploid and tetraploid plants could sometimes have the same length-breadth leaf index. *S. acaule* was characterized by smaller pollen size than *S. tuberosum*. The pollen grains of the former species as well as those of diploid species usually had three germination pores, whereas four-pored grains were common in *S. tuberosum*, *S. andigenum* and the artificial polyploids.

(12) The chromosome distribution of a selfed progeny of the pentaploid species *S. curtilobum* ($2n = 60$) deviated very much from the expected binominal distribution of plants with chromosome numbers ranging from 48 to 72. Plants with more than 62 chromosomes were not found, but some plants died before root-tips had been fixed. This observed distribution is somewhat unexpected in the light of the results obtained in certain $4x \times 5x$ and $5x \times 4x$ crosses. The leaves of *S. curtilobum* had two layers of palisade cells, just like *S. acaule*, but differing from most other species of the section *Tuberarium*.

(13) Secondary association was studied in diploid, triploid and tetraploid *Solanum* plants of different categories. The results of analysis of secondary association were too heterogeneous to prove that the original basic chromosome number of this genus is 6 or any other definite chromosome number less than 12. Most interesting was the observation that the degree of secondary association in asynaptic autotetraploid *S. Rybinii* was very low. Secondary association in *Solanum* thus seems to be due, at any rate partly, to a real attraction, probably caused by the same forces as give rise to the primary pairing.

(14) The chromosome distribution of the 3x × 2x cross *S. chaucha* × *S. stenotomum* and of the 3x × 4x cross *S. chaucha* × *S. tuberosum* was studied. In the former case there was a strong skewness in the diploid direction from the expected random distribution, in the latter case there was a deviation in the tetraploid direction. There was no indication of a secondary balance in favour of the assumed primary basic number 6. Meiosis in a double trisomic 2x + 1 + 1 plant is described. The aneuploid plants of these progenies in comparison with their euploid sibs showed a decreased pollen fertility and vigour that on an average was proportional to the degree of numerical unbalance.

(15) As mother *S. curtilobum* is easy to cross with *S. tuberosum* and *S. andigenum*. Here the 5x × 4x progenies consist of comparatively fertile $F_1$ hybrids. The 4x × 5x cross between *S. tuberosum* and *S. curtilobum* is difficult to perform, but was achieved by grafting the mother plant on tomato. The $F_1$ hybrids of this cross were completely male-sterile and also had a very reduced female fertility. Meiosis in the PMC's of the 5x × 4x hybrids was normal, in the 4x × 5x hybrids aberrant. The course of this aberrant meiosis is described. This dissimilar chromosome mechanism in the PMC's of these *Solanum* hybrids after reciprocal crossings may be due either to cytoplasmic inheritance or to maternal effects.

There were no significant differences between the distributions of somatic chromosome numbers among the hybrids of the reciprocal crosses. Hybrids with intermediate chromosome numbers were the most common. No correlation was found between chromosome number and plant vigour or chromosome number and pollen fertility. Here chromosomes in excess of 48 obviously have little effect on these properties. In the back-cross progeny of a 4x × 5x hybrid with 4x + 4 chromosomes to its 4x mother there was a skewness in the chromosome number distribution in the tetraploid direction.

(16) Crossings between the two tetraploid species *S. acaule* and

*S. tuberosum* failed in my experiments. After chromosome doubling of *S. acaule*, hybrids were easily obtained when this species was used as the female partner. The hexaploid and approximately hexaploid $F_1$ hybrids of this cross are described. These hybrids were fertile, and have been used for further matings.

(17) The nature of the differences between the diploid species of the section *Tuberarium* has been discussed. Our present knowledge of these species is, however, rather incomplete, and it is impossible to say whether these species are autogenomatic or not. As to the tetraploid species, the suggestion is made that *S. tuberosum* is mainly autotetraploid but relatively to the true artificial autotetraploids investigated somewhat differentiated in an allotetraploid direction. *S. acaule*, on the other hand, seems to be allotetraploid relative to *S. tuberosum*. Whether these two species are of intra- or inter-specific origin has not been settled. It has, however, been pointed out that there is no real evidence of the usually assumed hybrid origin of *S. tuberosum*.

(18) The assumed secondary polyploidy of the section *Tuberarium* is discussed from different points of view. As far as I can see, this assumption at present can neither be proved nor disproved, but none the less it seems to me likely that a chromosome number less than 12 was originally the basic one.

(19) The causes of incompatibility, including »one-way» incompatibility, within the section *Tuberarium* are discussed, and the probable importance of cytoplasmic conditions is stressed. This does not mean that the incompatibility should mainly be caused by cytoplasmic differences.

(20) The causes of total and partial sterility within the section *Tuberarium* are outlined. Modificatory sterility obviously plays an important rôle. Among the diploid allogamous species haplontic sterility caused by structural and numerical aberrations is probably common. The inquiry into the causes of the sterility of polyploid species has mainly been confined to *S. tuberosum*. Here diplontic sterility seems to be the most important. The absence of hypo- and hyper-tetraploids within this species is discussed. Here the probable importance of somatoplastic sterility has been pointed out, and a hypothesis is proposed which would account for the absence of such plants in *S. tuberosum* and for the various chromosome distributions obtained in selfings and inter-specific crosses of *S. curtilobum* and its derivatives.

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# ZYTOLOGISCHE STUDIEN AN MONOSOMISCHEN TYPEN VON GODETIA WHITNEYI

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(With a Summary in English)

DIE zytologische Untersuchung von *Godetia Whitneyi*-Pflanzen, die Dr. GUNNAR HIORTH, Ås, Norwegen, aus Samen aufgezogen hat, die er an natürlichen Standorten der Art eingesammelt hatte, hat recht interessante Ergebnisse gegeben. So wurden Monosomen aus Tocaloma, Marin Co., Cal., aus Santa Rosa 6, Sonoma Co., Cal., aus Horse Mtn, Humboldt Co., Cal., schliesslich aus Briceland in Oregon gefunden (HÅKANSSON, 1942). Sie hatten 13 Chromosomen und zeigten in der Metaphase 1 ein I. Die monosomischen Typen haben nach HIORTH gute Vitalität; es »liessen sich an keinen unserer 4 monosomen Typen abgesehen von herabgesetzter Fertilität irgendwelche Anzeichen von Schwäche oder Missbildungen feststellen, während ein gleichmässiger, kräftiger und harmonischer Wuchs stets notiert wurde» (HIORTH, 1944 a). Weitere Untersuchungen haben gezeigt, dass die Monosome S. Rosa 6 nach Kreuzung mit der Standardrasse Bremen viele Nullisomen, dass die Monosome Horse Mtn einige wenige Pflanzen mit 13 Chromosomen aber vollkommene Asyndese gibt, sowie dass die Monosome Briceland anscheinend konstant ist (HÅKANSSON, 1943 a).

HIORTH hat die Monosomen genetisch eingehend untersucht. Die vielen hierbei fixierten Pflanzen wurden von mir zytologisch studiert. Untersucht wurde die Nachkommenschaft von Monosomen, $F_1$ von Monosomen und Bremen, eine Rasse, die die Standardchromosomen 1.2,3.4,5.6,7.8,9.10,11.12,13.14 hat, schliesslich eine Kreuzung mit einer durch rezessive Gene gekennzeichneten Linie. Die Chromosomenpaarung kann nur in der Metaphase 1 studiert werden, die häufige Asyndese macht es aber in gewissen Fällen sehr schwierig, die typische Anordnung der Chromosomen festzustellen. Auch lassen sich einige Typen ziemlich schwer fixieren. Doch kann das untersuchte Material als recht gross bezeichnet werden, und viele Eigenschaften dieser Monosomen dürften geklärt sein. Die Chromosomenpaarung in Bastarden zwischen verschiedenen Monosomen ist aber noch nicht untersucht.

## MONOSOMEN AUS TOCALOMA.

Die Vererbung der Kotyledonenzeichnung Toc-*C*$^{pa}$ ist eigentümlich. Kreuzungen *C*$^{pa}$*c* × *cc* geben einen grossen Überschuss an *C*$^{pa}$, etwa 4,7 : 1 statt 1 : 1. In der Rückkreuzung (Toc-*C*$^{pa}$ × Bremen) × Bremen (Bremen ist *cc*) waren die beiden untersuchten *C*$^{pa}$-Pflanzen Monosomen. Die eine zeigte sehr starke Asyndese, doch konnten einige Male 4 o + 4 II + I festgestellt werden (S 1017—'41 a). Die zweite (b) zeigte nur schwache Asyndese, einige PMZ enthielten eine 4-Kette + 4 II + I; wahrscheinlich war dies die typische Anordnung. Häufiger sah man 6 II + I, ein II war aber stark heteromorph, die Kette war also vermutlich in zwei II aufgeteilt (HÅKANSSON, 1942). Wir werden finden, dass eine Asyndese in Toc-*C*$^{pa}$ sehr häufig ist, und die Feststellung der typischen Meiosisanordnungen sehr erschwert.

Die Bestimmung der Chromosomenzahl in Keimwurzeln zeigte, dass die Nachkommenschaft einer Monosome aus 13- und 14-chromosomigen Pflanzen bestand; die ersteren waren in grosser Mehrheit. Eine Untersuchung keimender Samen der Kreuzung Bremen ♀ × Toc-*C*$^{pa}$ ♂ lieferte auch Pflanzen mit 13 und 14 Chromosomen. Daraus geht hervor, dass nicht nur Eizellen sondern auch Pollenkörner mit 6 Chromosomen vital sind.

(*Toc-C*$^{pa}$ ♀ × *Bremen* ♂), *s.* — 2 *C*$^{pa}$-Pflanzen aus S 126—'42 enthielten Meiosisstadien. G 85 war eine Monosome mit der Chromosomenanordnung eine 4-Kette + 4 II + I. Ein Ring kam anscheinend nie vor. Die Anordnung war leicht festzustellen, da diese Pflanze keine Asyndese zeigte. G 88 war disomisch und zeigte ausgesprochene Asyndese. So wurden 5 II + 4 I, 4 II + 6 I, 3 II + 8 I beobachtet; es gab aber nicht viele deutliche Teilungen. Die typische Anordnung war wohl sicher eine 4-Kette + 5 II (siehe unten). Es kommen also *C*$^{pa}$-Pflanzen mit 14 Chromosomen vor.

*Bremen* ♀ × *Toc-C*$^{pa}$ ♂. — Es kommen *cc*- und *C*$^{pa}$ *c*-Pflanzen vor; sie bilden nach HIORTH eine Art Zwillingbastarde mit stark verschiedenem Habitus. Zwei untersuchte *cc* (S 130—'42) hatten 7 II. Eine Asyndese unter Bildung von 2 I kam nur selten vor. Die *C*$^{pa}$-Pflanzen (S 128—'42) dieser Kreuzung waren meist zu spät fixiert. In der EMZ der Pflanze G 93 konnte jedoch eine 4-Kette + 4 II + I festgestellt werden. Eine andere Pflanze hatte Teilungen in den PMZ, die Anordnung schien eine 4-Kette + 5 II zu sein. Eine *C*$^{pa}$-Pflanze aus einer anderen Kreuzung Bremen ♀ × *C*$^{pa}$ ♂ (S 130—'42) war eine

Monosome mit starker Asyndese (ähnlich S 1017 a). Fig. 3 zeigt 2 II + 9 I.

Aus verschiedenen Gründen konnten hier nur wenige Pflanzen zytologisch klargelegt werden, gewisse Schlüsse können jedoch gezogen werden. Die 7 II in *cc* zeigen, dass die benutzten *C*pa-Pflanzen den Standardkomplex *a* enthielten. Die 4-Kette der *C*pa-Pflanzen zeigt, dass sie eine 4-Konfiguration hatten. Ihr zweiter Komplex ist also strukturell verschieden von *a*, er kann *g* genannt werden. Die Elimination des I, das ja ein *a*-Chromosom ist, bringt es mit sich, dass sehr oft 6-chromosomige Gonen gebildet werden. Die Monosome bildet vier verschiedene Gonen. Diese sind *a* mit 6 und mit 7 sowie *g* mit 6 und mit 7 Chromosomen. Die bisherigen Ergebnisse zeigten nun, dass von diesen Gonen folgende vital sind: 7-chromosomiges *a* und *g* und 6-chromosomiges *g*. Dies gilt für Embryosäcke; ob 7-chromosomige *g*-Pollenkörner funktionieren ist noch fraglich.

*Toc-C*pa. $S_2$. — Die *C*pa-Pflanzen waren aus Kreuzungen mit Bremen hervorgegangen und hatten also sicher einen *a*-Komplex. Nach HIORTH (brieflich) kommen hier folgende *C*pa-Typen vor. M. Satter, mittelgrosser Zentralfleck auf dem Kronblatt, frühe Blühzeit, relativ schmalblättrig, wahrscheinlich monosom. R. Stark reduzierter Zentralfleck, Blätter breiter, später. K. Kleinblütig, ohne Fleck auf dem Kronblatt, meistens oder stets hochgradig pollensteril; ein dominanter Typus, der ziemlich selten auftritt. G. Nullisom? Niedrig, buschig, spät. Fruchtknoten lang. Kronblattfleck sehr gross (partiell pollensteril). Erinnert etwas an die Nullisomen von Santa Rosa 6, aber mit besserer Pollenfertilität.

Nachdem nun bekannt ist, dass wenigstens drei vitale Gonentypen gebildet werden, ist es nicht überraschend, dass in der Nachkommenschaft von *C*pa recht verschiedene Typen vorkommen.

Der *Typus R* wurde in den Familien S 395—'43 und S 397—'43 studiert. Er ist disomisch mit der Anordnung 4-Kette + 5 II. Die Familie S 395 zeigte nicht den üblichen Überschuss an *C*pa, ihre Spaltung war 131 *C*pa + 145 *c*, die Elternpflanze war also höchstwahrscheinlich nicht monosom sondern hatte 14 Chromosomen. Von den 6 untersuchten Pflanzen war auch nur eine (G 708) monosomisch mit einer 4-Kette + 4 II + I. Die fünf Disomen hatten auch alle eine 4-Kette. Ein Ring wurde nie beobachtet. Die Kette scheint in allen *C*pa-Pflanzen dasselbe Aussehen zu haben (Fig. 5). Ein Endchromosom ist auffallend lang: es scheint das grösste Chromosom des Kerns zu sein. Sein Nachbar ist ziemlich gross und heterobrachial; er hat recht

ungleichgrosse Arme. Dann kommt ein recht grosses Chromosom mit submedianem Zentromer. Das zweite Endchromosom ist auffallend kurz. Die Lage der Zentromeren der Endchromosomen kann nicht ermittelt werden. Ein interstitielles Chiasma wurde in der Kette nie beobachtet. Die Kettenchromosomen waren beinahe immer im Zickzack angeordnet.

Eine gewisse, jedoch schwache Asyndese kam vor. Vielleicht war sie in verschiedenen Pflanzen ungleich stark. In G 711 war die häufigste Anordnung 6 II + 2 I, dann 5 II + 4 I, und schliesslich eine 4-Kette + + 5 II. Die Asyndese traf also meistens die Kettenchromosomen. In G 712 war die typische Anordnung die häufigste, die Kette schien selten aufgeteilt zu sein. In einem Präparat von G 707 wurde gezählt: typische Anordnung in 11,6 II + 2 I in 7,3-Kette + 4 II + 3 I in 4,3-Kette + + I + 5 II in 1,5 II + 4 I in 2,7 II in 1 PMZ (Fig. 6). Extra Univalente können in der Anaphase längsgeteilt werden. S 708 (die monosomische Pflanze) zeigte keine Asyndese.

S 397 war die Nachkommenschaft der oben erwähnten Pflanze G 88/126—'42. Diese war ja eine Disome mit starker Asyndese. Die Spaltung war 42 $C^{pa}$ : 27 *c*. 4 fixierte $C^{pa}$-Pflanzen hatten 14 Chromosomen und zeigten durchweg sehr starke Asyndese. Die typische Anordnung 4-Kette + 5 II kam jedoch in einer Anzahl PMZ vor. Auffallend war nun die Bildung von Univalenten. So kamen in den PMZ von G 717 von 0 bis 14 I vor. Die I lagen zu jeder Seite der Äquatorialplatte wie die Homologen von Bivalenten (Fig. 7). Man bekam hierdurch den Eindruck, dass die Chiasmen erst spät aufgelöst werden, wenn die II schon gegen die Pole orientiert sind. Hiermit stimmt die in der Anaphase mässige Elimination überein; nur I-e, die in der Äquatorialebene liegen, werden häufiger eliminiert. - Immerhin sah man in G 717 häufig 1 oder 2 eliminierte I in der Interkinese oder in der 2. Teilung. Die anderen Pflanzen hatten kaum eine so starke Asyndese, 8—10 I konnten jedoch beobachtet werden. Man vergleiche damit S 395, wo selten mehr als 4 I vorhanden waren. Wir haben in S 397 eine Art gleitende Asyndese, die alle Stufen von vollständiger Syndese bis zu vollständiger Asyndese aufweisen kann (Fig. 8).

*Der Typus M* kam in S 394—'42 vor. Diese Familie war die durch Selbstbefruchtung erhaltene Nachkommenschaft der Pflanze G 85/126—'42, die eine Monosome ohne Asyndese war. Drei M-Pflanzen hatten eine 4-Kette + 4 II + I (Fig. 2). Die Kette sah wie bei den Disomen aus. Die Asyndese war sehr schwach. Das I liegt in der Äquatorialebene. Es wird verspätet und häufig eliminiert (Fig. 1).

Fig. 1—16. *Godetia Whitneyi* Tocaloma. 1—4 monosomische C$^{pa}$ (*a.g*). 1: Anfang der zweiten Teilung, das I ist eliminiert. — 2: eine 4-Kette + 4 II + I. — 3: eine asyndetische Pflanze, 2 II + 9 I. — 4: Non-disjunction in der Kette. — 5—8 disomische C$^{pa}$ (*a.g* + 1). 5: eine 4-Kette + 5 II. — 6: 7 II, zwei sind heteromorph. — 7: asyndetische Pflanze, eine 3-Kette + 2 II + 7 I. — 8: asyndetische Pflanze, 14 I. — 9—11: Typus K, 9 Disome, 10, 11 Trisomen. 9: 4 II + 6 I. — 10: 6 II + 3 1. — 11: Anaphase 1. — 12—16 *C*$^{pa}$ *c* $W_3 \times cc\ w_3w_3$. 12: *C*$^{pa}$ $w_3$, eine 3-Kette + 3 II + 4 I. — 13: *C*$^{pa}$ $w_3$, eine 4-Kette + 4 II + I. — 14: *c* $W_3$, 7 II. — 15: *c* $w_3$, 6 II + 1, 2 II sind heteromorph. — 16: *c* $w_3$, Anaphase 1, Teilung von I-en.

Eine Elimination dürfte in etwa 70 % der PMZ vorkommen. Das I wurde in Anaphase 2 geteilt.

In der Familie S 394 gab es eine Variante, die Pflanze G 706. Sie hatte aber dieselbe Anordnung wie der Typus M. Das I wird aber hier entschieden öfter eliminiert, die Frequenz betrug etwa 85 %. Es ist aber ungewiss, ob dieser Tatsache Bedeutung zukommt, vielleicht war die hohe Elimination nur zufällig.

*Der Typus G* war für die Familie S 393—'43 charakteristisch. Es war nicht leicht Meiosisstadien zu finden. G ist keine Nullisome; die Chromosomenzahl ist 13. In zwei Pflanzen konnte die Anordnung festgestellt werden; sie war eine 4-Kette + 4 II + I. Die Kette war wie beim Typus M; dasselbe gilt für die Elimination. Die Asyndese war schwach ausgeprägt.

Vom *Typus K* wurden die Pflanzen G 2771, 2772 und 2773 studiert. 2771 (aus S 392—'43) hatte 14 Chromosomen, ihre meiotischen Teilungen waren abgeschlossen. Überzählige Kerne oder Zellen zeigten aber, dass eine ausgeprägte Asyndese mit Elimination vorgekommen ist. 2772 (aus S 393) zeigte nur wenige deutliche Teilungen. Wahrscheinlich kann eine 4-Kette vorkommen, dies konnte aber nicht sicher festgestellt werden. Die Anordnung 4 II + 6 I wurde beobachtet (Fig. 9) und Elimination kommt vor. Etwas besser konnte 2773 (S 401—'43) studiert werden. Die Chromosomenzahl betrug hier 15. Ein III wurde beobachtet; Univalente kamen oft vor. Sie zeigten eine Neigung, sich in der Äquatorialebene einzuordnen (Fig. 10). Dies führt dazu, dass die I verspätet werden und dass eine starke Elimination folgt. In der Teilung 2 gab es in beinahe jeder PMZ eliminierte Chromosomen. Fig. 11 zeigt fünf Chromosomen in der Äquatorialplatte, während die Pole der Spindel 5 bzw. 5 haben. Das Vorkommen einer 4-Kette ist wahrscheinlich, konnte aber nicht sicher festgestellt werden.

Es ist nun ganz klar, dass die Toc-$C^{pa}$ durch eine 4-Kette mit Endchromosomen recht verschiedener Grösse gekennzeichnet ist. Die Kette hat zwei *a*-Chromosomen, während zwei, darunter wahrscheinlich das grosse Endchromosom, dem speziellen Komplex *g* angehören. $C^{pa}$ muss seinen Locus in einem *g*-Chromosom der Kette haben. Die Konstitution der Monosomen ist *a.g.* *g* ist mit 6 oder 7 Chromosomen vital, *a* nur mit 7. Disomische und monosomische Toc-$C^{pa}$ sind morphologisch verschieden (Typus R bzw. M). Ihre Spaltung ist verschieden, weil sie bei den Disomen durch keine grosse Elimination von einem I beeinflusst wird; ihre Konstitution ist *a.g* + 1. Trotzdem 6-chromosomige männliche und weibliche Gonen vital sind, kommen Nullisomen nicht vor.

Dies muss auf zygotischer Letalität beruhen. Der G-Typus hatte eine Zytologie, die mit der von M die grösste Ähnlichkeit zeigte, und die Deutung dieses abweichenden Typus, der nach HIORTH nur in bestimmten Teilen des $C^{pa}$-Stammbaumes spaltet, ist ungewiss. Die Zytologie des Typus K schliesslich konnte nicht vollständig geklärt werden. Die Meiosis war offenbar in allen Pflanzen stark gestört mit starker Elimination von Univalenten. Die Ursache dieser Elimination scheint eine Neigung der I zu sein, sich in die Platte einzuordnen. Die grosse Pollensterilität des K-Typus hat so eine Erklärung gefunden. Die eine K-Pflanze war eine Trisome, zwei hatten 14 Chromosomen. Die Trisome verdankt ihre Bildung einem Non-disjunction. Die 14-chromosomigen K-Pflanzen sind vermutlich in ähnlicher Weise entstanden, nur die 8-chromosomige Non-disjunction-Gone ist mit einer 6-chromosomigen *g*-Gone verschmolzen.

$C^{pa} \times w_3w_3$. — Über diese Kreuzung hat HIORTH berichtet (HIORTH, 1944 a). $w_3$ bedingt u. a. weisse Grundfarbe der Blüten. Toc-$C^{pa}$ hat das normale Allel $W_3$. Die $w_3w_3$-Pflanzen hatten keine Kotyledonenzeichnung. Die Kreuzung ist also $\frac{C^{pa}\ W_3}{c\ \ -} \times \frac{c\ w_3}{c\ w_3}$ und in $F_1$ sind 1) $\frac{C^{pa}\ W_3}{c\ \ w_3}$, 2) $\frac{C^{pa}\ w_3}{c\ \ -}$, 3) $\frac{c\ W_3}{c\ w_3}$ und 4) $\frac{c\ w_3}{c\ -}$ zu erwarten, wenn die $C^{pa}$-Pflanze monosomisch war, und $W_3$, wie die Ergebnisse der Kreuzung zeigen, im I lokalisiert war. Es waren nämlich die meisten Pflanzen $w_3$, das rezessive Merkmal trat schon in $F_1$ zutage. Die Phänotypen kamen in folgender Anzahl vor:

$$18\ C^{pa}\ W_3 : 264\ C^{pa}\ w_3 : 23\ c\ W_3 : 1\ c\ w_3.$$

Der grosse Überschuss an $C^{pa}$ zeigt, dass eine Monosome zu der Kreuzung verwendet wurde. Recht viele Pflanzen dieser wichtigen Kreuzung wurden fixiert. Brauchbare Teilungen waren aber nicht in allen vorhanden.

$C^{pa}\ W_3$: (S 409, S 410 A—'43). Eine Pflanze (G 742) hatte 15, 5 hatten 14 Chromosomen. Die Meiosis konnte in drei studiert werden. Die Anordnung war eine 4-Kette + 5 II. Fig. 4 zeigt eine Orientierung der Kettenchromosomen, die zu Non-disjunction führen muss. Zwei Pflanzen aus S 409 zeigten kaum Asyndese, die Pflanze aus S 410 A hatte aber recht häufig I-e.

$C^{pa}\ w_3$: (S 408 A, 410 A—'43). Die fixierten Pflanzen hatten 13 Chromosomen. Eine Meiosis wurde nur in drei angetroffen und war sehr schwierig zu studieren. Die Fixierung war nicht gut und Asyndese

kommt vor. Die häufigste Anordnung war 6 II + I. Schliesslich konnten aber in allen drei Pflanzen einige gut fixierte PMZ ohne Asyndese gefunden werden. Fig. 13 zeigt eine typische 4-Kette + 4 II + I. Fig. 12 zeigt, dass das kleine Endchromosom frei ist. Nähere Studien wurden nicht gemacht.

*c* $W_3$: (S 408 B). Drei Pflanzen hatten 7 II (Fig. 14).

*c* $w_3$: (S 410 B). Die Blütenknospen sind grösser als bei $C^{pa}$ $w_3$ und die Fixierung ist weit besser ausgefallen. Eine falsche Klassifizierung ist also unwahrscheinlich. Die Anordnung der Chromosomen ist aber dieselbe wie bei $C^{pa}$ $w_3$. Die Anordnung eine 4-Kette + 4 II + + I konnte mehrmals festgestellt werden. Recht häufig kamen 6 II vor, eines war stark, eines schwach heteromorph (Fig. 15). Infolge von Asyndese kamen auch andere Anordnungen vor: 5 II + 3 I, 4 II + 5 I, 3 II + 7 I, eine 3-Kette + 4 II + 2 I, eine 3-Kette + 3 II + + 4 I. In der Anaphase wurden bisweilen I-e eliminiert (Fig. 16).

Das Ergebnis der zytologischen Untersuchung bestätigt die von HIORTH vorgelegte Deutung dieser Kreuzung. Die zu der Kreuzung verwendete $C^{pa}$-Pflanze war monosomisch. Sie bildete 6-chromosomige *g*- und *a*-Gonen in grossem Überschuss. Die ersteren sind vital und veranlassen die Bildung der vielen $C^{pa}$ $w_3$-Pflanzen (*a.g*). Die letzteren sind letal. Zwar hatte eine einzige Pflanze die genetische Konstitution *c* $w_3$, was darauf hindeutete, dass sie aus 7- und 6-chromosomigen *a* entstanden ist. Ihre Zytologie zeigte aber, dass sie *a.g* war, weshalb man vermuten kann, dass dies beruht darauf, dass $C^{pa}$ in seltenen Fällen infolge von Crossing-over sich vom *g*-Komplex entfernt hat. Viel seltener werden von der Monosome 7-chromosomige *g* und *a* gebildet. Die erstere Gonenklasse bedingt die Bildung von $C^{pa}$ $W_3$ (*a.g* + *1*), die letztere von *c* $W_3$ (*a.a*); sie sind etwa gleich häufig. Nur scheint die Elimination des I nicht so häufig zu sein wie die Zahlenverhältnisse dieser Kreuzung andeuten. Beträgt die Elimination 70 %, dann haben von den *g*-Gonen 70 % + 15 % 6, 15 % 7 Chromosomen. Man sieht, dass eine Elimination von 85 %, wie wir sie in einer Variante fanden, besser zu den gefundenen Spaltungszahlen passt. Wahrscheinlich ist doch, dass die Elimination in den Samenanlagen grösser ist als in den PMZ.

Die Disomen der Kreuzung sind $W_3$, die Monosomen $w_3$. Dies muss darauf beruhen, dass $W_3$ seinen Locus im I der Mutterpflanze hat. HIORTH bezeichnet dieses Chromosom mit 5.6. Mit $w_3$ stark gekoppelt sind *kr* (Krone auf schmale Streifen reduziert) und *sp* (spitze Narben). HIORTH fand aber, dass die Kreuzungen von Toc-$C^{pa}$ mit *kr*

und *sp* in $F_1$ nicht die rezessiven Merkmale zeigen: $w_3$ zeigt Pseudodominanz, nicht aber *kr* und *sp*, die auch zum Chromosom 5.6 gehören. Dies muss darauf beruhen, »dass die 6-chromosomigen *g*-Gameten die normalen Allele für *sp* und *kr* enthalten, obgleich diese sonst in dem diesem Komplex fehlenden Chromosom 5.6 zu suchen sind» (HIORTH, l. c.). Sie haben in Toc-$C^{pa}$ ihre Loci in einem anderen Chromosom als normal, vermutlich im *g*-Teil der Kette, während $W_3$ auch in Toc-$C^{pa}$ seinen Locus in 5.6 hat.

Das Fehlen der Anordnung 6 II + I in *c* $W_3$ zeigt überzeugend, dass 6-chromosomige *a*-Gonen nie vital sind. Beobachtungen über Non-disjunction in der Kette liegen aber vor. Die häufige Asyndese muss bisweilen auch Non-disjunction verursachen. Es wurden ja auch Trisomen im Material gefunden, die für die Vitalität 8-chromosomiger Gonen sprechen.

Es sind noch zwei Familien mit Toc-$C^{pa}$ untersucht worden. Neue Ergebnisse haben diese Untersuchungen nicht geliefert; sie sollen jedoch kurz besprochen werden.

*S 471—'43.* — Zwei monosomische $C^{pa}$-Pflanzen wurden untersucht. G 770 zeigte keine Asyndese, ihr I wurde in $^2/_3$ der PMZ eliminiert, G 769 zeigte ausgeprägte Asyndese. Es konnten bis 5 nachhinkende I beobachtet werden. Geschwisterpflanzen können also in bezug auf den Grad der Asyndese sehr verschieden sein.

*S 435—'43.* — Die meisten untersuchten $C^{pa}$ waren aus Kreuzungen mit Bremen hervorgegangen, hatten aber das Zytoplasma von Tocaloma. Nur in zwei Fällen hatten sie Bremen-Zytoplasma (Kreuzung Bremen ♀ × $C^{pa}$ ♂). Drei $C^{pa}$-Pflanzen mit Bremen-Zytoplasma aus S 435 zeigten Asyndese, oft sah man nur II und I. Bisweilen kam doch eine 4-Kette vor. Die Chromosomen waren auffallend gross und man bekam den Eindruck, dass Bivalente und Kettenchromosomen im Bremen-Zytoplasma grösser sind als in dem von Tocaloma. Etwa gleichgrosse Chromosomen habe ich aber auch in gewissen Pflanzen mit Tocaloma-Zytoplasma gefunden. Die Verhältnisse sind also nicht gerade einfach. — In Wurzelmitosen sind allerdings die Chromosomen von $C^{pa}$ × *c c* ebenso gross wie die von *c c* × $C^{pa}$ *c*.

*S 428—'43.* — [Bremen × ($C^{pa}$ × Bremen)], s. Diese Familie spaltete $^1/_4$ Zwerge aus. Einige solche wurden fixiert, zeigten aber leider keine Meiosisstadien. Mitosen in jungen Blumenblättern zeigten in zwei Pflanzen 13 Chromosomen. In einer dritten wurden aber nur 12 gezählt. Vielleicht sind die Zwerge *g.g*, die verschiedenen Zahlen könnten dann darauf beruhen, dass bei der Bildung zwei der Pflanzen eine

7-chromosomige *g*-Gone teilgenommen hat. Es ist aber klar, dass von dieser Familie neue Fixierungen untersucht werden müssen.

*C*$^{pa}$ *C*$^{s}$ *c*. — S 350—355—'42 waren [(*Toc*-*C*$^{pa}$ × Bremen) × (*C*$^{s}$ × Kew AB)] × Bremen, also (*C*$^{pa}$ × *C*$^{s}$) × *c c*. Die verschiedenen Familien zeigten dieselbe Spaltung, so gab S 350 75 *C*$^{pa}$ : 12 *C*$^{s}$ : 1 *C*$^{pa}$ *C*$^{s}$. Analoge Kreuzungen mit anderen Kotyledonenzeichnungen haben immer gezeigt, dass die seltenen Pflanzen mit zwei verschiedenen Kotyledonenzeichnungen, die in Rückkreuzungen zu *cc* auftreten, durch Non-disjunction gebildet worden sind. Man könnte dies auch hier vermuten.

Drei *C*$^{pa}$ *C*$^{s}$-Pflanzen wurden untersucht. Sie hatten 14 Chromosomen. In den PMZ war immer ein I vorhanden. Die häufigste Anordnung der Chromosomen war eine 5-Kette + 4 II + I. Dies ist wohl sicher die typische Anordnung (Fig. 17). Bisweilen wurde eine 4-Kette + + 4 II + 2 I beobachtet (Fig. 18), häufiger war aber eine 3-Kette + + 5 II + I (Fig. 19). Offenbar handelte es sich um Non-disjunction, das man sich in folgender Weise denken kann.

Die 4-Kette von *C*$^{pa}$ kann mit 1.2—2.3—3.4—4.1 bezeichnet werden, das Univalent ist 5.6. *C*$^{pa}$ hat, wie angenommen wird, seinen Locus im Chromosom 2.3, *C*$^{s}$ im 3.4. Non-disjunction der Kette gab eine Gone mit den Chromosomen 2.3—3.4—4.1. Sie hat also *C*$^{pa}$ und *C*$^{s}$. Mit einer *a*-Gone 1.2, 3.4, 5.6 wird eine Pflanze gebildet, die die gefundenen Anordnungen zeigen muss, z. B. die 5-Kette 3.4—4.3—3.2—2.1—1.4 und das I 5.6. Die beiden 3.4 Chromosomen haben *C*$^{s}$ bzw. *c*. Die Anordnung mit einer 3-Kette wäre: 4.1—1.2—2.3 + 5.6 + 3.4—4.3. Ein *a*-Chromosom (mit *C*$^{s}$) folgte in der Mutterpflanze den *g*-Chromosomen der Kette beim Non-disjunction; so können also die Beobachtungen erklärt werden.

Wir haben schliesslich zwei Erscheinungen der Toc-*C*$^{pa}$ zu erörtern, die durch die kombinierten genetischen und zytologischen Untersuchungen keine Erklärung gefunden haben. Es ist erstens die Tatsache, dass die 4-Konfiguration nie geschlossen ist, und zweitens die häufige Asyndese. Die Asyndese veranlasste die Bildung von I-en, die Auflösung von Chiasmen geschah aber offenbar spät, weshalb die Anaphasenstörungen in den meisten Fällen mässig waren. Restitutionskerne wurden nicht gebildet. In gewissen Fällen wurden oft recht viele I-e gebildet, in anderen dagegen stets nur wenige. In gewissen Familien zeigten die untersuchten Pflanzen alle etwa dieselbe Asyndese, die mit der der selbstbestäubten Mutterpflanze übereinstimmte. In anderen Fällen waren Pflanzen mit verschiedener Asyndese in der-

selben Familie vorhanden. Die Asyndese ist offenbar genisch bedingt. Es sind aber zu wenig *cc*-Pflanzen untersucht, um Schlüsse über den Erbgang der Asyndese ziehen zu können.

In der Kette gehören ein auffallend grosses und ein recht grosses Chromosom dem einem, ein recht grosses und ein kleines dem anderen Komplex an. Dass die Konfiguration offen ist, und dass sie, wie es scheint, recht leicht in zwei offene II zerfällt, kann seine Erklärung darin finden, dass die reziproke Translokation nur kürzere Stücke traf. Die Kette hat konstantes Aussehen, was bedeutet, dass immer dasselbe Chiasma aufgelöst wird.

## MONOSOMEN UND NULLISOMEN AUS SANTA ROSA 6.

Dieser Fundort liegt 3 miles südöstlich der Stadt Santa Rosa, Sonoma Co., Cal. 4 Pflanzen hatten hier $4_0 + 5$ II gezeigt, 1 Pflanze war monosomisch (HÅKANSSON, 1942). In $F_2$ der Kreuzung S. Rosa Monosome × Bremen war in grosser Individuenzahl ein auffälliger, stark steriler Typus aufgetreten. Dieser erwies sich als eine Nullisome mit starker Asyndese; die Metaphase 1 zeigte in der Regel 12 I (HÅKANSSON, 1943 a).

*Monosome.* — Die Familie S 594—'43, die Kreuzung einer vermuteten Monosome mit Bremen, war ein sehr ungünstiges Material. Drei Pflanzen wurden studiert. Sie hatten eine 4-Kette mit verschieden grossen Endchromosomen. Ein Ring wurde nicht sicher beobachtet. Das I wird häufig eliminiert. Eine sehr schwache Asyndese ist beobachtet worden, sodass bisweilen zwei neue I-e vorkommen können. Unter den fixierten Pflanzen der Familie S 664 waren neben Nullisomen einige Monosomen. Dieselben Beobachtungen wurden hier gemacht. Asyndese kommt kaum vor. Die Elimination des I erfolgt etwa wie bei Tocaloma. Sein Zentromer scheint sich aber hier früher zu teilen, bisweilen schon in der Anaphase 1, häufiger in der Interkinese, doch oft erst in der Anaphase 2. Das I war selten durch einen Faden mit einem II verbunden. Die Konstitution der S. Rosa-Monosomen ist *a.g*, doch ist es unbekannt, ob die Kette von denselben Chromosomen gebildet wird wie bei Tocaloma. Das Vorhandensein des *a*-Komplexes ergibt sich daraus, dass die Anordnung in der Monosome und in Monosome × Bremen dieselbe ist.

*Zwei disome Varianten.* — S 601—'43 war die Kreuzung S. Rosa 6 × Bremen $F_2$, von der hier zwei Varianten fixiert worden sind. Sie hatten 14 Chromosomen; die Anordnung in der Metaphase 1 war hier

nicht so konstant. In G 734 konnte eine 4-Kette + 5 II festgestellt werden; die Endchromosomen der Kette waren von recht verschiedener Grösse (Fig. 25). Nicht selten wurden 6 II + 2 I und 7 II beobachtet und mehrere I konnten vorkommen, die Störungen in der Anaphase verursachten (Fig. 24). G 733 zeigte stärkere Störungen. Vermutlich ist die typische Anordnung auch hier eine 4-Kette + 5 II; sie konnte

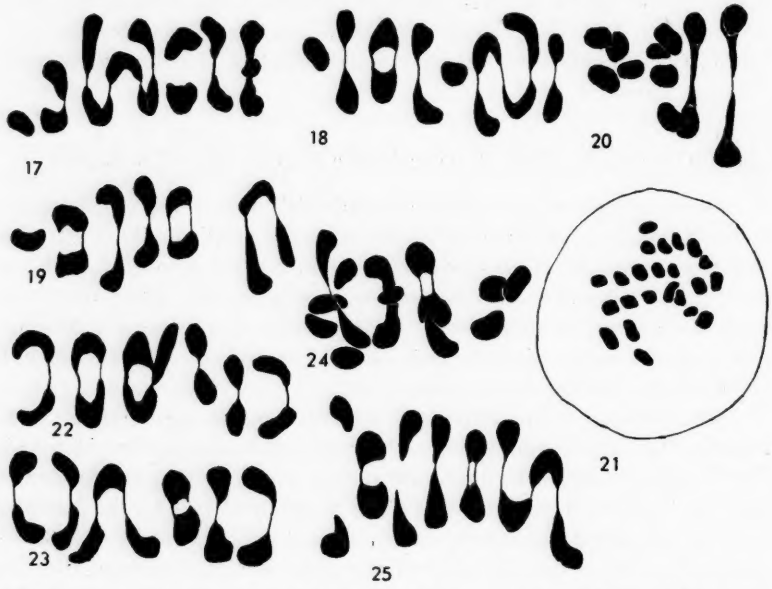


Fig. 17—25. *G. Whitneyi.* 17—19 Tocaloma $C^{pa}$ $C^{s}$ *c*. 17: eine 5-Kette + 4 II + I. — 18: eine 4-Kette + 4 II + 2 I. — 19: eine 3-Kette + 5 II + I. — 20—25 Santa Rosa 6. 20: Nullisome (*g.g*) 2 II + 8 I. — 21: Nullisome, eine »tetraploide» PMZ 23 I. — 22—23: abweichende Monosome (*g.g* + 1) 5 II + III. — 24—25: eine disomische Variante (*a.g* + 1). 24: 3 II + 8 I. — 25: eine 4-Kette + 5 II.

aber kaum beobachtet werden. Eine 3-Kette kam aber oft vor, so z. B. wurde eine 3-Kette + 3 II + 5 I beobachtet.

Diese Varianten sind aus einer 7-chromosomigen *g*-Gone und eine *a*-Gone von Bremen gebildet. Die Störungen waren hier viel auffallender als in den Monosomen.

*Nullisomen.* — Die ersten Nullisomen wurden in $F_2$ der Kreuzung von Bremen mit der Mutterpflanze von S 662—'43 festgestellt. In einer Nachkommenschaft, erhalten durch Selbstung dieser Mutterpflanze, kamen auch Nullisomen vor. Zwei untersuchte Pflanzen hatten dieselbe

Zytologie, die die nach Kreuzung erhaltenen Nullisomen zeigten. In der Regel ist die Asyndese vollständig, nur ziemlich selten wurde 1 II + 10 I beobachtet. Restitutionskerne werden recht häufig gebildet; die Häufigkeit der Diaden betrug etwa 30 %.

Auch in der Nachkommenschaft anderer geselbsteter S. Rosa 6-Pflanzen wurden Nullisomen festgestellt. Sie gehen offenbar regelmässig und in grosser Anzahl aus Monosomen hervor. Den ersten nullisomischen Typus beschreibt HIORTH (1944 a): »Diese Nullisomen mit 12 Chromosomen sind zwar verspätet, kleinblütig und haben herabgesetzte Grösse; dennoch ist ihre Wuchs durchaus harmonisch und abgesehen von Sterilität zeigen sie keinerlei Missbildungen, sondern machen eher den Eindruck einer neuen Art». Die aus Inzuchtlinien hervorgegangenen Nullisomen sind etwa vom selben Typus, gewisse Unterschiede kommen jedoch vor. So zeigten einige Pflanzen eine schwache Fertilität, und der nullisomische Typus der Familien S 663—'43 und S 669—'43 wurde als relativ kräftig bezeichnet. Ausserdem wurden Nullisomen in S 664—'43 und S 665—'43 gefunden.

Die Zytologie der verschiedenen Pflanzen war aber sehr ähnlich. Einige bildeten ein II sehr selten, andere in beinahe 50 % der PMZ, diese Verschiedenheit wurde aber schon in dem früher untersuchten Material beobachtet. Einmal wurden 2 II gefunden (Fig. 20). Die II waren immer von gleich grossen Chromosomen gebildet, Heteromorphie wurde nicht beobachtet. Einmal wurde eine sehr grosse PMZ gefunden, die verdoppelte Chromosomenzahl hatte. 23 I konnten sicher festgestellt werden (Fig. 21). Die Asyndese war also, wie erwartet, auch in »tetraploiden» PMZ vollständig. Die Frequenz der Restitutionskerne war auch verschieden. Eine Pflanze hatte entschieden weniger Diaden als die früher untersuchten Nullisomen, nur etwa 10 %, mehrere Pflanzen hatten aber mehr. Eine Frequenz von 50 % kann vorkommen. Verschiedene Antherenfächer derselben Knospe können aber ganz verschiedene Diadenfrequenz zeigen, sie ist sicher leicht modifikativ beeinflussbar.

Es kommt also in Santa Rosa 6 anscheinend nur ein ganz bestimmter nullisomischer Typus vor. Die Variation der Fertilität des Pollens dürfte modifikativ sein. Man kann vermuten, dass nur unreduzierte Pollenkörner fungieren. Von HIORTH ist nun durch neue Kreuzungen festgestellt worden, dass alle Nullisomen nicht vollständig steril sind; ein Teil der Eizellen und Pollenkörner sind fertil.

*Eine monosomische Variante.* — S 665—'43 ist die durch Selbstung erhaltene Nachkommenschaft der Pflanze 2117/597—'40. Es waren

hier nur wenige normale Pflanzen vorhanden. Unter den »Zwergen» waren nach HIORTH zwei Typen, einer mit dunkelgrüner Blattfarbe, aber mit zum Teil hellem Blattrand (dies soll Nullisomie wahrscheinlich machen), und einer mit gelblichgrüner Blattfarbe. Unter den untersuchten »Zwergen» waren vier gewöhnliche Nullisomen. Zwei waren aber Monosomen. Von diesen konnte G 1140 näher studiert werden, während G 1139 schlechter fixiert war, aber wahrscheinlich dieselbe Zytologie hatte.

Bei dieser Monosome kommt kein konstantes I vor. Die Anordnung ist in der Regel 6 II + III. Das III zeigt oft V-Form (Fig. 23); »ring-and-rod» wurde aber auch beobachtet (Fig. 22). Die Konfiguration entspricht also einem wahren III. Die häufige Elimination eines I, die in gewöhnlichen Monosomen Regel ist, beobachtet man also hier nicht. Doch dürften etwa 20 % der PMZ in der Interkinesis oder in Teilung 2 ein eliminiertes I haben. Störungen in der Anaphase 1 sind nämlich recht häufig. 5 II + III wurden in G 1139 in einem Diakinesekern beobachtet. — Eine interessante Beobachtung war die folgende: In einem Kleinfach einer Anthere zeigten die PMZ vollständige Asyndese, sie hatten alle 13 I.

*Allgemeines.* — Wie andere Monosomen bilden die aus S. Rosa 6 viele Gonen mit 6-chromosomigen *a* und *g*, relativ wenige mit 7-chromosomigen *a* oder *g*. Die ersterwähnte Gonensorte ist wohl sicher wie bei Tocaloma steril, jedenfalls spricht nichts dagegen. Die Ergebnisse der Kreuzungen, d. h. die Bildung disomischer Varianten mit einer 4-Kette und das Auftreten von Normalpflanzen beweisen die Fertilität der anderen Gonensorten. Die Nullisomen dürften alle *g.g* sein. Ihre Frequenz ist sehr gross, so war das Verhältnis in einer Bremen-Kreuzung 34 »Zwerge» : 48 »Nicht-Zwerge». Die Einheitlichkeit des Typus macht es aber unwahrscheinlich, dass auch *a.g* oder gar *a.a*-Nullisomen vorkommen. Wie hervorgehoben wurde, sind die II, die von der Nullisome gebildet werden, immer homomorph. Dies deutet darauf hin, dass sie eine homozygotische Kombination darstellt, also nicht *a.g* sein kann.

Die monosomische Variante ist vielleicht schwieriger zu deuten. Die Chromosomenanordnung spricht dafür, dass sie trisomisch ist, und eine homozygotische Kombination darstellt. Ihre Trisomie zeigt, dass sie nicht die Kombination 7-chromosomiges *g*. 6-chromosomiges *g* ist. Gewisse nullisomische Eigenschaften machen aber die Deutung als *g.g* wahrscheinlich. Man kann sich die Bildung in folgender Weise vorstellen. Durch Non-disjunction in der 4-Kette folgt ein *a*-Chromosom

den beiden *g*-Chromosomen. Die dadurch gebildete Non-disjunction-Gamete befruchtet ein 6-chromosomiges *g*. Die gebildete Zygote ist also eine Nullisome mit einem extra *a*-Chromosom, das nicht das I der Monosomen sondern ein Kettenchromosom ist. Auffallend ist, dass die gebildete Form nicht asyndetisch ist. Die Asyndese der nullisomischen *g.g* wird durch das Vorhandensein eines *a*-Chromosoms aus der Kette aufgehoben (vergl. S. 154).

## MONOSOMEN AUS HORSE MTN.

Zwei monosomische Pflanzen wurden schon früher gefunden (HÅKANSSON, 1942). Die eine hatte eine 4-Kette und zeigte einige Male eine Brücke in der Anaphase 1, die andere schien keine Kette zu haben, konnte aber nur wenig studiert werden. Zwei Bastardpflanzen Horse Mtn × Bremen mit recht hoher Pollensterilität waren monosomisch, wenigstens die eine hatte eine 4-Kette. Später wurde über $F_2$ dieser Kreuzung berichtet (HÅKANSSON, 1943 a). 3 $F_2$-Pflanzen hatten eine 4-Kette + 4 II + I, eine mit sehr dünnen Antheren (S 663 a—'42) hatte 14 Chromosomen, sehr häufig eine Asyndese mit 2 oder 4 I aber keine Kette. In allen $F_2$-Familien spaltete in wenigen % ein hochgradig steriler Typus mit grünem statt rotem Stengel und kleinen Blüten aus. Der letztgenannte hatte 13 Chromosomen, zeigte aber immer vollständige Asyndese und bildete sehr häufig (zu etwa 80 %) Restitutionskerne.

Nur wenig neues Material ist untersucht. Es ist aber jetzt klar, dass die Monosomen durch eine ähnliche Kette wie die Tocaloma-Monosomen ausgezeichnet sind. Ihre Chromosomen scheinen dieselben zu sein. Eine gewisse Asyndese kann vorkommen; sie und die Untersuchung eines zu kleines Materials ist sicher die Ursache, dass die Kette in einigen Fällen bisher übersehen wurde. S 589—'43 war die Nachkommenschaft der Kreuzung Bremen ♀ × Monosome ♂. Monosomische Pflanzen in $F_1$ zeigen, dass 6-chromosomige Pollenkörner vital sind.

S 584—'43 war die Nachkommenschaft nach Kreuzung mit Bremen der Pflanze mit dünnen Antheren. Die Pflanzen hatten aber normale Antheren und waren Monosomen. Besonders in einer Pflanze war Asyndese häufig; es wurden bis 5 I beobachtet. Oft waren daneben nur II, aber in einigen PMZ konnte auch eine typische 4-Kette festgestellt werden. Dies zeigt, dass die Mutterpflanze auch eine 4-Kette hatte, die doch nicht beobachtet worden ist. Die Mutterpflanze war also keine homozygotische Kombination.

Unter den Nachkommen der Horse Mtn-Monosomen gibt es also

Monosomen (*a.g*), Normalpflanzen (*a.a*) und die Pflanze mit dünnen Antheren (*a.g* + 1). Nullisomen kommen offenbar nicht vor. Der eigentümliche kleinblütige Typus mit 13 I kann nun in einer anderen Weise als früher gedeutet werden. Ich glaubte, dass das I der Monosomen bisweilen unter »Misdivision» des Zentromers geteilt wird, wodurch ein Isochromosom mit zwei gleichen Armen entsteht. Der neue Typus sollte dieses Chromosom besitzen und also in bezug auf einen Chromosomenarm nullisomisch sein. Dies könnte die Asyndese erklären. Ich glaubte nämlich, die Pflanze mit dünnen Antheren sei die Kombination *g* + 1.*g* + 1. Dies ist, wie hervorgehoben wurde, nicht der Fall. Der kleinblütige Typus kann also sehr wohl *g.g* + 1 sein, also aus 7-chromosomigem *g* und 6-chromosomigem *g* gebildet worden sein. Die Ähnlichkeiten mit der Nullisome aus Santa Rosa 6 können auch in dieser Weise erklärt werden. *g.g* sollte wie in S. Rosa 6 sich durch völlige Asyndese auszeichnen. Weitere Untersuchungen müssen entscheiden, ob der kleinblütige Typus ein modifizierter *a.g* oder *g.g* ist. Die letztere Deutung harmoniert besser mit den Ergebnissen, die sonst über die Zytogenetik der *Whitneyi*-Monosomen gewonnen wurde. Misdivision ist in *G. Whitneyi* zwar beobachtet, genetische Folgen davon sind aber, von dem hier erörterten Fall abgesehen, nicht bekannt.

## MONOSOMEN AUS BRICELAND.

Es wurden einige Pflanzen aus Samen vom natürlichen Standort untersucht. Drei hatten 4 0 + 5 II, eine 6 0 + 4 II, eine 4 0 + 4 II + I. Monosomen kommen also in der Natur vor.

*C*sp. — HIORTH hat die Genetik von zwei Kotyledonenzeichnungen eingehend studiert (HIORTH, 1944 b), *C*sp hat seinen Locus im veränderten Komplex in einem 6-Ring (sp = Sprenkelung). Bei der Kreuzung mit Bremen (*cc*) ist $F_1$ normal, ihre Rückkreuzung mit Bremen gibt *C*sp *c* mit normalen Blüten und 6 0, und *cc* mit stark defekten Blüten und 7 II (HÅKANSSON, 1942). Ein Bremen-Chromosom stört in homozygotischem Zustand die Entwicklung der Blüten, wenn die Pflanze Zytoplasma von Briceland hat, nicht aber wenn sie Bremen-Plasma hat. Neue *C*sp-Pflanzen wurden jetzt untersucht (sie waren alle von einem neuen Stammbaum B). Sie sind aus der Nachkommenschaft geselbsteter *C*sp nach Kreuzung mit Bremen entstanden. Die Familie S 137 A—'42 spaltete nach 235 *C*sp : 20 *c*, S 138—'42 106 *C*sp : 0 *c*. Die untersuchten *C*sp hatten alle 6 0 + 4 II; der Ring konnte bisweilen offen oder in zwei Ketten aufgeteilt sein. Die *c*-Pflanzen hatten

defekte Blüten. Die Spaltung von $C^{sp}$ ist oft eigentümlich: Nach HIORTH wird $C^{sp}$ durch den Pollen meist im Überschuss vererbt, »fast zu 100 %», es »hat jedoch ein Teil der Familien niedrigere Prozente bis herab zu 50 %». Pflanzen aus noch einer Familie wurden untersucht. S 494—'43 stammt aus der Kreuzung Bremen ♀ × $C^{sp}$ ♂, hatte also Zytoplasma von Bremen; die Spaltung war hier 262 $C^{sp}$ : 205 *c*, und die *c*-Pflanzen waren hier normal entwickelt. $C^{sp}$ hatte auch hier 6 I + 4 II.

In der Nachkommenschaft aus Selbstung von $C^{sp}$ $C^{st}$ sind nach HIORTH Homozygoten ($C^{sp}$ $C^{sp}$). Drei wurden studiert, sie hatten 7 II. In einem II waren die Chromosomen bisweilen nur lose vereint oder traten als zwei I auf (Fig. 28). Ich habe dies in anderen »Strukturhomozygoten» beobachtet, z. B. in $C^{s}$ $C^{s}$ in Bremenplasma (HÅKANSSON, 1944 a) oder besonders ausgeprägt in einer homozygotischen interspezifischen Translokation (HÅKANSSON, 1944 b). $C^{sp}$ $C^{sp}$ ist also *b.b*, wenn mit *b* der veränderte Komplex bezeichnet wird.

$C^{st}$. — Bisher sind nur zwei $C^{st}$-Pflanzen (st = Streifung des Stieles der Kotyledonen) studiert worden; sie waren Monosomen (HÅKANSSON, 1942). Die eine war gut fixiert und zeigte einen 4-Ring oder eine 4-Kette und I. Das I war gross und wurde in der Anaphase 1 geteilt.

Die weiteren Untersuchungen haben die Monosomie von $C^{st}$ bestätigt. S 149—'42 ist $C^{st}$ geselbstet. S 540—'43 ist eine Kreuzung S 149—'42 × Bremen und hat Briceland-Zytoplasma, das eine Reduktion der Grösse der Antheren bedingt. S 549—'43 stammt auch aus Kreuzungen mit Bremen, hat aber Bremen-Zytoplasma, die Blüten sind bei diesen normal entwickelt. Alle untersuchten $C^{st}$ waren Monosomen und strukturelle Heterozygoten. Den früher behandelten Monosomen gegenüber zeigten $C^{st}$ folgende Unterschiede: 1) Die 4-Konfiguration ist oft geschlossen (Fig. 26). 2) Das I wurde noch häufiger eliminiert. 3) Asyndese kommt beinahe nie vor. Ein Bastard $C^{st}$ $C^{pa}$ wurde auch untersucht. Leider fehlten Meiosisstadien, sodass nicht festgestellt werden konnte, ob die 4-Konfiguration von denselben Chromosomen gebildet wird wie in Tocaloma.

Es ist etwa ebenso oft eine Kette wie einen Ring vorhanden. Die Chromosomen in der Mitte der Kette sind recht gross, haben ein submedianes Zentromer, das eine Endchromosom ist klein, das andere gross, doch ist es anscheinend nicht so auffallend wie in Tocaloma. Ein bestimmtes Chiasma scheint sich also zu öffen. Ein winziges Fragment wurde in einer einzigen Anthere beobachtet. Das I teilte sich nicht

in der Anaphase 1. Die im Jahre 1942 untersuchte Pflanze war also in dieser Hinsicht nicht typisch. Es teilt sich in der Teilung 2; selten unterbleibt diese Teilung. In wenigstens 90 % der PMZ wird das I während der Anaphase 1 eliminiert.

Das Verhalten der Chromosomen in den Samenanlagen wurde auch beobachtet. Mehrmals wurde die Elimination des I festgestellt (Fig. 32 und 33). Nähere Studien wurden nicht gemacht, aber es ist klar, dass die Mehrzahl der Eizellen wie die der Pollenkörner 6 Chromosomen haben.

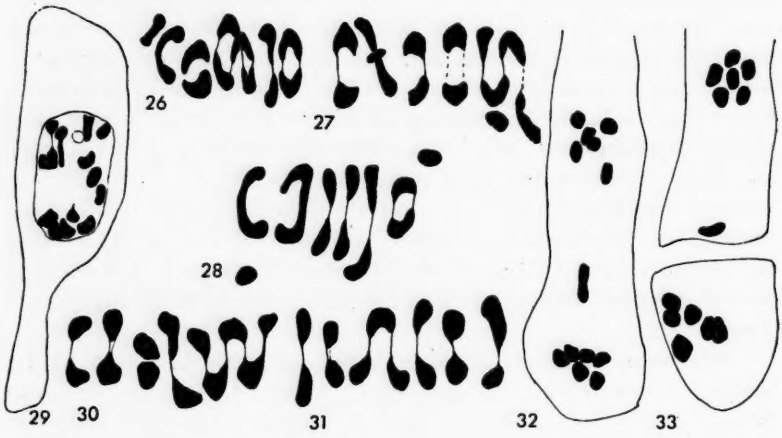


Fig. 26—33. *G. Whitneyi*, 27 Dyerville, sonst Briceland. 26: Bric-$C^{st}$, 4 0 + 4 II + I. — 27: Dyerville-*cc*, eine 4-Kette + 4 II + I. — 28: Bric-$C^{sp}$ $C^{sp}$, 6 II + 2 I. — 29: $C^{s}$ *c* aus $C^{st}$ *c* × $C^{s}$ *c*. Abnorme PMZ. Diakinese, 2 II + 11 I. — 30—31: $C^{st}$ $C^{sp}$, 30: eine 5-Kette + 4 II. — 31: eine 3-Kette + 5 II, ein II stark heteromorph. — 32—33: $C^{st}$, Meiosis in der Samenanlage, 32: Anaphase 1. — 33: Anfang der zweiten Teilung, das I ist eliminiert.

$C^{st}$ hat ohne Zweifel seinen Locus in einem *g*-Chromosom des Ringes. Der *g*-Komplex ist hier ausserordentlich stark bevorzugt. Die Kreuzungen $C^{st}$ *c* ♀ × *cc* ♂ (Bremen) hatten unter 1108 Nachkommen nur 2 *cc*. Die letzteren waren missbildete Pflanzen und hatten wahrscheinlich nicht 14 sondern 15 Chromosomen (siehe unten). Dass nur Monosomen gebildet werden, kann kaum durch die Elimination des I erklärt werden, wenn diese auch sehr gross ist. HIORTH (1944 b) hat zu zeigen versucht, dass dies auf einer Hemmung der *cc*-Embryonen in $C^{st}$ *c*-Müttern beruht. Man könnte sich vorstellen, dass Trisomie das

Hemmungssystem beeinflussen und vitale *cc* veranlassen kann. Eine Selbstung von $C^{st}$ *c* gab 56 Nachkommen, die alle $C^{st}$ waren.

In der Kreuzung *cc* ♀ × $C^{st}$ *c* ♂ wurden aber viele *cc*-Pflanzen gebildet, in der Nachkommenschaft betrugen sie 25 % (HIORTH, l. c.). Dies ist überraschend viel; nach der grossen Univalentenelimination sollte man weniger erwarten. Irgend ein Faktor muss hier das numerische Defizit an *a*-Gonen teilweise kompensieren. $C^{st}$-Homozygoten sind nicht bekannt; sie sind offenbar nicht vital. Nullisomen wurden nicht gefunden. Schliesslich hat HIORTH gefunden, dass in $C^{st}$ ♀ × Bremen schon in $F_1$ sämtliche Pflanzen defekte Blüten haben. Sie sind jedoch nicht so stark missbildet wie die *cc*-Pflanzen aus $C^{sp}$ *c* × *cc*. Sie haben immerhin reduzierte Antheren und überzählige Griffel. In der monosomischen $C^{st}$ übt also das betreffende Bremen-Chromosom schon in heterozygotischem Zustand in Briceland-Plasma einen Einfluss auf die Ausbildung der Blüten aus.

$C^{st}$ *c* × $C^{s}$ *c*. — Die Kreuzungen waren ($C^{st}$ × Bremen) × ($C^{s}$ × Kew AB), sie gaben 77 $C^{st}$ $C^{s}$ : 77 $C^{st}$ *c* : 1 $C^{s}$ *c*. Es war also nur eine einzige Pflanze nicht $C^{st}$. Diese Pflanze, G 149/153—'42, wurde untersucht. Die Blüten waren missbildet, es gelang jedoch einzelne Kleinfächer zu finden. Sie waren recht abnorm: Die Tapetenzellen waren übernormal gross, im selben Fach liegende PMZ konnten stark verschiedene Stadien zeigen. Einige PMZ waren stark verlängert (Fig. 29). In Mitosen wurden 15 Chromosomen gezählt, leider konnte infolge starker Asyndese die Chromosomenbindung nicht näher studiert werden. Anzeichen einer Ringkonfiguration gab es nicht und es wurde auch keine erwartet. Fig. 29 zeigt 2 II + 11 I. Disomische Nicht-$C^{st}$ wurden also in der Kreuzung nicht gebildet, man kann also vermuten, dass auch in $C^{st}$ *c* × *cc* die beiden *cc* nicht disomisch, sondern trisomisch sind.

$C^{st}$ $C^{sp}$. — Durch Kreuzung ist die Kombination *b.g* hergestellt worden. Die Pflanzen zeigten beide Kotyledonenzeichnungen; untersucht wurden drei aus S 143—'42 und drei aus S 547—'43.

Sie waren Monosomen, hatten aber kein I. In der Regel ist die Anordnung 5 II + eine 3-Kette (Fig. 31). Die Endchromosomen der Kette sind etwa gleich gross. Die Kette ist als V orientiert und ihre Endchromosomen gehen nach demselben Pol. Selten folgt eine andere Verteilung statt: Wenn ihre Chromosomen in einer Reihe geordnet sind, dann gehen die Endchromosomen nach verschiedenen Polen. Von den II ist eines stark heteromorph. Die strukturelle Heterozygotie ist also grösser als das Vorkommen einer 3-Kette anzeigen kann. So wurde in S 547 eine 5-Kette in einigen PMZ beobachtet. Ein Endchromosom

dieser Kette war auffallend klein (Fig. 30). Dies besagt, dass das grössere Chromosom des heteromorphen II:s ein Chiasma mit einem Endchromosom der 3-Kette gebildet hat. Die typische Anordnung ist also 4 II + eine 5-Kette, aber in der Regel ist ein Chiasma in der Kette aufgelöst.

$C^{st}$ muss in $C^{st}$ $C^{sp}$ im Mittelchromosom der 3-Kette liegen, während $C^{sp}$ in einem Endchromosom seinen Locus hat. Nach Selbstung werden etwa $^1/_4$ $C^{sp}$ $C^{sp}$ und $^3/_4$ $C^{st}$ $C^{sp}$ gebildet. Die ersteren haben 7 II und werden von zwei 7-chromosomigen, die letzteren von einer 7- und einer 6-chromosomigen Gone gebildet. Die weiblichen Rückkreuzungen $C^{st}$ $C^{sp}$ × *cc* gaben nach HIORTH (l. c.) in 4 Familien 2 $C^{st}$ $C^{sp}$ *c* : 253 $C^{st}$ *c* : 1 $C^{sp}$ *c* : 0 *cc*. Es kommen hier beinahe keine $C^{sp}$ *c*-Pflanzen vor. Ihr Ausbleiben kann ja nicht auf Elimination eines I beruhen, denn ein solches gibt es in den Mutterpflanzen ja nicht. Diese Möglichkeit zur Erklärung des Ausbleibens von 14-chromosomigen Pflanzen gab es in $C^{st}$ *c*, kann aber hier nicht in Betracht kommen. In beiden Fällen sterben nach HIORTH wahrscheinlich die 14-chromosomigen Zygoten ab. Die wenigen $C^{st}$ $C^{sp}$ *c*-Pflanzen sind wohl durch eine abweichende Verteilung der Kettenchromosomen entstanden (vgl. oben).

*Allgemeines.* — Die gefundenen Bindungen können in folgender Weise erklärt werden. $C^{st}$ hat den Ring 1.2—2.3—3.4—4.1 und das I 5.6. Es ist *a.g*, und von vitalen Gonen werden meist 6-chromosomige *g*-Gonen mit 2.3 und 1.4 aber ohne 5.6 gebildet. $C^{sp}$ *c* hat den 6-Ring 1.2—2.3—3.4—4.5—5.6—6.1, der *b*-Komplex ist 2.3, 4.5, 1.6. Bei diesen Bezeichnungen kann man in $C^{st}$ $C^{sp}$ (*b.g*) folgende Anordnung erwarten: Die 3-Kette 5.4—4.1—1.6 und das II 3.2—2.3. Diese Anordnung wurde ja in der Regel gefunden. Die starke Heteromorphie in einem II zeigt aber, dass das Chromosom 2.3 in den beiden Komplexen verschiedene Grösse hat. Selten wurde eine Kette 3.2—2.3—5.4—4.1—1.6 mit einem kleinen Endchromosom gebildet. Das Chromosom 2.3 von *g* kann in seltenen Fällen ein Chiasma mit dem Ende .5 (oder mit dem Ende .6?) der Kette bilden. Das Chromosom 2.3 von *b* ist also kleiner als 2.3 von *g*.

Man könnte erwarten, dass auch in $C^{st}$ bisweilen ein solches Chiasma gebildet wird. Dies sollte bedeuten, dass in der Monosome das I bisweilen mit der Kette vereint war. Dies wurde aber nicht sicher beobachtet. Möglich ist, dass diese Chiasmabildung in $C^{st}$ $C^{sp}$ darum leichter stattfindet, weil die grössere Konfiguration (die 3-Kette) ganz verschiedene Enden hat, die miteinander kein Chiasma bilden können.

Sind die Enden gleich, dann wird in erster Linie zwischen ihnen ein Chiasma gebildet.

Die Genetik von Briceland-$C^{st}$ is also komplizierter als die der anderen Monosomen. Ausser der durch die Elimination des I bedingten Verschiebung der Spaltungszahlen, wirken hier zytoplasmatische Unterschiede, z. B. Bremen gegenüber, die sich in Missbildung der Blüten äussern, und schliesslich ein Hemmungssystem, das die *cc*-Embryonen auf $C^{st}$-Mütter hemmt. Diese Monosomen sind deshalb auch in Bremen-Kreuzungen konstant, in denen sonst zwei zytologische Typen von einer konstanten Heterozygote wie Kew AB bedingt werden (HÅKANSSON, 1942).

## MONOSOMEN AUS DYERVILLE.

Früher wurde aus Dyerville, Humboldt Co., Cal., nur eine Pflanze Dyerville × Bremen untersucht. Sie hatte 4 ○ + 5 II; ein Ringchromosom war auffallend klein. Es wurden nun drei Pflanzen der Kreuzung Dy-$C^{st}$ × Bremen untersucht. Sie waren Monosomen. Die Zytologie ähnelte der von Briceland $C^{st}$. Es waren aber die *cc*-Pflanzen, die diese Zytologie zeigten.

Die 4-Konfiguration ist eine Kette oder ein Ring; eine Kette ist häufiger. Drei Kettenchromosomen zeigen etwa dieselbe Grösse, eines war aber entschieden kleiner. Der Ring ist also dem früher gefundenen ähnlich. Das I wurde in etwa $^3/_4$ der PMZ eliminiert. Es schien kleiner als bei den anderen Monosomen zu sein. Sein Zentromer wurde oft schon in der Interkinese geteilt.

Wie HIORTH brieflich mitteilte, ist ihre Genetik eine ganz andere als die von Bric-$C^{st}$. Die hier studierte Familie S 608—'43 zeigte die Spaltung 53 *cc* : 2 $C^{st}$. Die fixierten Pflanzen waren *cc*. Der Locus von Dy-$C^{st}$ kann nicht in *g* liegen, er muss sich in einem *a*-Chromosom des Ringes befinden.

## DIE SOMATISCHEN CHROMOSOMEN.

Chromosomenmorphologische Studien in *G. Whitneyi* sind sehr schwierig (vgl. HÅKANSSON, 1942). Beobachtungen über Wurzelmitosen und die Meiosis von Haploiden zeigen, dass 3 von den sieben Chromosomen ein submedianes Zentromer, 2 ein subterminales und 2 das Zentromer zwischen Mitte und Ende des Chromosoms haben. Der Platz des Zentromers ist in den beiden letztgenannten nicht derselbe. 2 von diesen Chromosomen können Satelliten haben.

Fig. 34 zeigt eine Wurzelmitose von *G. Whitneyi* Bremen × *G. Bottae* (vgl. HÅKANSSON, 1943 b). Die Bremen-Chromosomen sind früher nicht abgebildet. Die Chromosomen sind hier leichter zu studieren, da die *Whitneyi*-Chromosomen nämlich viel grösser sind als die *Bottae*-Chromosomen; man sieht also den haploiden Satz. Die neuen Beobachtungen bestätigen die früheren. So ist von den Chromosomen mit submedianem Zentromer eines grösser als die anderen, die ihrerseits verschiedene Grösse haben. In der abgebildeten Platte befindet sich nur ein SAT-Chromosom, der Satellit ist sehr klein und wird von einem Chromosom mit subterminalem Zentromer proximal getragen. Ein zweiter Satellit konnte hier nicht beobachtet werden.

Die Mitosen der Monosomen liessen die Lage des Zentromers in den Chromosomen in der Regel nicht erkennen. Die Satelliten konnten jedoch beobachtet werden. Fig. 35 zeigt eine Kernplatte der Monosome

Fig. 34—37. Somatische Kernplatten. 34: *G. Whitneyi* Bremen × *G. Bottae*, 7 grosse *Whitneyi*- + 9 kleine *Bottae*-Chromosomen. — 35: Tocaloma-$C^{pa}$. — 36: monosomische Santa Rosa 6. — 37: nullisomische Santa Rosa 6.

Toc-$C^{pa}$. Man kann das Vorhandensein von vier SAT-Chromosomen feststellen. Bemerkenswert ist, dass ein Chromosom grösser als die anderen erscheint. Dies muss das grosse Endchromosom der Kette sein, das also grösser ist als die Chromosomen des grössten II:es. Ein Chromosom hat in dieser Platte ein schwach gefärbtes Endsegment.

Auch die Monosome Santa Rosa 6 hat ein Chromosom, das etwas länger als die Chromosomen des grössten II:es zu sein scheint (Fig. 36). Die entsprechende Nullisome hat ihrerseits zwei Chromosomen, die grösser als die anderen sind (Fig. 37). Dies muss das grosse Kettenchromosom sein, das in der Nullisome zweimal vertreten ist. Hierdurch wird klar, dass dieses Chromosom dem *g*-Komplex angehören muss, was ja schon früher wahrscheinlich erschien. Das kleine Endchromosom ist somit ein *a*-Chromosom. In Santa Rosa 6 wurden nur 2 SAT-Chromosomen beobachtet.

Die Monosome Horse Mtn. schliesslich hatte auch ein Chromosom, das deutlich grösser als die anderen war. Es hat sein Zentromer

zwischen Mitte und Ende wie auch aus einigen Beobachtungen an Santa Rosa 6 und Tocaloma hervorzugehen scheint (Fig. 36).

## ALLGEMEINER TEIL.

Die Untersuchungen eines grossen Materials haben nicht zur Auffindung einer einzigen Pflanze mit 6 II + I geführt. Die Monosomen von *Godetia Whitneyi* sind offenbar strukturelle Heterozygoten mit der Anordnung $4_0 + 4$ II + I. Drei der monosomischen Haupttypen hatten eine 4-Kette, zwei hatten einen Ring oder eine Kette. Die Monosomie scheint durch die 4-Konfiguration in irgend einer Weise kompensiert zu sein. Es wurde aber nie eine Verbindung zwischen dem I und der Kette beobachtet, reziproke Translokationen zwischen dem Chromosom, das ein I ist, und den Kettenchromosomen sind also anscheinend nicht vorgekommen. In allen Typen hatten die Endchromosomen der Kette sehr verschiedene Grösse. Von Santa Rosa 6, Horse Mtn und Tocaloma-$C^{pa}$ wurde in Mitosen gezeigt, dass das grosse Endchromosom das grösste Chromosom des Kerns war. Erst die Untersuchung von Bastarden kann aber entscheiden, ob die 4-Konfiguration in den verschiedenen Monosomen von denselben Chromosomen gebildet wird. Zytologische Unterschiede zwischen einerseits Briceland-$C^{st}$ und Dyerville-*cc*, andererseits den drei anderen Typen scheinen aber vorzukommen. Das genetische Verhalten der Monosomen ist allerdings stark verschieden.

Der spezielle Komplex der Monosomen wird hier *g* genannt (HIORTH hat $\gamma$). Der zweite Komplex ist der Standardkomplex *a* (HIORTHs $\alpha$). Die 4-Konfiguration wird vorläufig mit 4.1—1.2—2.3—3.4 bezeichnet und das I mit 5.6. *g* hat die Chromosomen 1.4, 2.3, 7.8, 9.10, 11.12, 13.14, das Chromosom 5.6 fehlt in der Regel, *g* hat nur 6 Chromosomen. Die Beobachtungen über die somatischen Chromosomen zeigten, dass das grosse Endchromosom der Kette ein *g*-Chromosom sein muss. Hier soll erwähnt werden, dass wir in *G. Whitneyi* ausser dem Standardkomplex *a* und dem nullisomischen Komplex *g*, noch zwei andere Arten von Komplexen haben. Der eine ist *b* (von HIORTH $\beta$ genannt), der mit Standard Chromosomenringe gibt. Es gibt viele verschiedene *b*-Komplexe in *Whitneyi* (HÅKANSSON, 1942 und unpubliziert), Ringe mit 4, 6, 8 oder 10 Chromosomen sind gewöhnlich, und es ist gezeigt worden, dass z. B. die 6-Ringe in Briceland-$C^{sp}$ und Kew AB nicht von denselben Chromosomen gebildet werden. Eine vierte Art von *Whitneyi*-Komplex

wurde *d* (HIORTHS $\delta$) genannt. Durch *d* wird mit *a* die Anordnung 5 II + eine 3-Kette + I hervorgerufen (HÅKANSSON, 1944 a).

In der Meiosis der Monosomen tritt eine starke Elimination des I ein. Es werden weit mehr 6-chromosomige als 7-chromosomige Gonen gebildet. Die Elimination betrug in den PMZ im allgemeinen 70 %, in Briceland-$C^{st}$ noch mehr. Gonen mit *a* oder *g* + 1 sind im Defizit, mit *g* und *a* — 1 im Überschuss. Die Untersuchungen der Kreuzung $C^{pa}$ *c* $W_3 \times$ *cc* $w_3w_3$ haben es ausser Zweifel gestellt, dass *a* — 1 Gonen steril sind. Die Erfahrungen an anderen Monosomen und an Pflanzen mit einer 3-Kette und I (HÅKANSSON, 1944 a) zeigen dasselbe: Der Standardkomplex muss vollständig sein um eine fertile Gone zu geben. Die erwähnte Kreuzung zeigte unter den Eizellen monosomischer $C^{pa}$ 86 % *g*, 6 % *g* + 1 und 7 % *a*.

Die reziproken Kreuzungen mit Bremen haben wie erwartet Pflanzen mit 7 II (*a.a*), mit 4 o + 4 II + I (*a.g*) und 4 o + 5 II (*a.g* + 1) gegeben. Dies wurde betreffs Tocaloma, Santa Rosa 6 und Horse Mtn. festgestellt. Monosomen aus Briceland verhielten sich aber anders. Hier wurden in der weiblichen Rückkreuzung nur Pflanzen mit *a.g* gebildet (nebst zwei missbildeten Pflanzen, die wahrscheinlich Trisomen waren). Dies beruht nach HIORTH sehr wahrscheinlich darauf, dass die *a.a*-Embryonen hier absterben; *a.g* + 1 sind von Briceland nicht bekannt.

In der Nachkommenschaft von Monosomen sind theoretisch folgende Kombinationen zu erwarten: 1) Die Nullisome *g.g* (sollte die häufigste sein), 2) *g.g* + 1 (sollte recht häufig sein), 3) die gewöhnliche Monosome *a.g* (sehr häufig), 4) *a.g* + 1 und 5) *a.a*. Die beiden letzten Kombinationen sollten selten sein. Es hat sich aber gezeigt, dass die verschiedenen Monosomen ganz verschiedene Nachkommenschaft geben.

*g.g* wird nur von Santa Rosa 6 ausgespalten. Die Nullisome ist ein bestimmter sehr charakteristischer Typus; sie hat morphologische Eigenschaften, die den Gedanken auf eine neue Art lenken, ist aber stark steril. Sie ist sehr asyndetisch, die gewöhnliche Anordnung ist 12 I. Das Fehlen von Nullisomen in der Nachkommenschaft der anderen Monosomen muss darauf beruhen, dass diese Zygoten letal sind, da sowohl die ♂- wie ♀-Gonen mit 6 Chromosomen vital sind. In Santa Rosa 6 wird die Nullisome wie erwartet in grosser Anzahl gebildet.

*g.g* + 1 wurde wahrscheinlich von Horse Mtn.-Monosomen gebildet. Sie bilden in geringer Individuenzahl einen stark sterilen Typus mit kleinen Blüten und grünem statt rotem Stengel, der an die Nulli-

some von Santa Rosa erinnert. Er zeigte immer 13 I und bildete ausserordentlich häufig Restitutionskerne (HÅKANSSON, 1943 a). Man sollte den Typus auch in Santa Rosa erwarten, er ist aber dort bisher noch nicht gefunden worden. Vielleicht ist er der Nullisome zu ähnlich um leicht entdeckt zu werden.

*a.g* + 1 ist der disomische $C^{pa}$-Typus in Tocaloma, der nach Selbstung der Monosome oder in ihrer Kreuzung mit Bremen auftritt. Er hat bestimmte morphologische Eigenschaften und ist mit Typus R von HIORTH identisch. Die Spaltung disomischer $C^{pa}$ ist eine andere als die der monosomischen; einen grossen Überschuss an $C^{pa}$-Pflanzen gibt es in seiner Nachkommenschaft nicht. Auch in Santa Rosa 6 und Horse Mtn. kommen »Varianten» vor, die dieser Kombination entsprechen, wenn auch eine störende Asyndese die Feststellung der typischen Chromosomenanordnung erschwert.

In der Nachkommenschaft sind jedoch die Mehrzahl der Pflanzen Monosomen (in Santa Rosa 6 neben Nullisomen). Dies ist auch in Kreuzungen zwischen Monosomen und Bremen der Fall. Ein Extremfall liegt in Briceland-$C^{st}$ vor. Dies ist eine anscheinend konstante Monosome und ihre Bastardierungen mit Bremen sind auch monomorph. Eine strukturelle Heterozygote vom Typus *a.g* hat somit Konstanz erworben. Früher wurden in *G. Whitneyi* konstante Strukturheterozygoten vom Typus *a.b* in den Gartenrassen Kew A und Kew AB (und noch eine dritte Rasse) gefunden. Sie hatten alle 6 ∘ + 4 II, ihre Bremen-Bastarden waren zytologisch dimorph, sie hatten 6 ∘ oder 7 II. Schliesslich kennen wir eine konstante Strukturheterozygote vom Typus *a.d*. Es ist dies Tocaloma-$C^{pc}$ (HÅKANSSON, 1944 a). Der *d*-Komplex ist hier stark bevorzugt. $C^{pc}$ hat eine 3-Kette + I, ist konstant, spaltet aber einzelne letale gelbe Pflanzen ab. In Kreuzungen mit Bremen gibt es einen gewaltigen Überschuss an $C^{pc}$.

Dies war die normale Variation der monosomischen Typen, die sich also als ganz verschieden zeigen. Es ist aber auch Non-disjunction und Crossing-over in der 4-Kette festgestellt worden. Die Kettenchromosomen sind selten in einer Weise orientiert, die zu Non-disjunction führt (Fig. 4). Das Ergebnis haben wir in der Kreuzung $C^{pa}\ C^{s} \times cc$ gesehen (S. 138). Hier kamen einzelne $C^{pa}\ C^{s}\ c$ vor; diese können ja genetisch durch Non-disjunction entstanden sein, und zytologisch besteht kein Zweifel, dass sie in der geschilderten Weise gebildet sind, da sie die Chromosomenzahl und -paarung zeigen, die dann zu erwarten sind. Eine monosomische Variante aus Santa Rosa ist sehr wahrscheinlich in dieser Weise entstanden (s. u.). Schliesslich haben wir den

K-Typus von Tocaloma, der mit 14 und 15 Chromosomen vorkommen kann, und recht starke, durch Elimination bedingte Sterilität zeigt. Er ist wahrscheinlich zu den Non-disjunction-Pflanzen zu zählen.

Crossing-over haben wir sehr wahrscheinlich in der Kreuzung $C^{pa}$ *c* $W_3 \times$ *cc* $w_3w_3$ gefunden. Es kam hier eine einzige *c* $w_3$-Pflanze vor. Diese hatte aber dieselbe Chromosomenanordnung wie die $C^{pa}$-Monosomen der Kreuzung. Hier muss *c* seinen Locus vom *a*-Teil des Ringes mit $C^{pa}$ vom *g*-Teil ausgetauscht haben, was wohl durch Crossing-over geschah.

Einige Monosomen zeigten eine andere Anordnung der Chromosomen als die von den natürlichen Standorten stammenden Typen. Eben erwähnt wurde die Monosome mit vollkommener Asyndese, die aus gewöhnlichen Monosomen von Horse Mtn. in geringer Anzahl entsteht. Zwei Monosomen hatten kein I. Die durch Kreuzung hergestellte $C^{st}C^{sp}$ aus Briceland hatte 5 II + eine 3-Kette oder, selten, 4 II + eine 5-Kette; die Bedeutung und Genetik dieser ist S. 148 behandelt. Von Santa Rosa 6 kennen wir schliesslich zwei Pflanzen mit 5 II + III. Es wurde vermutet, dass diese Pflanzen *g.g* + 1 seien, aber das 13:te Chromosom war nicht das I 5.6 der Monosome, sondern ein *a*-Chromosom der Kette. Eine solche Konstitution erklärt nämlich die Anordnungen in den betreffenden Pflanzen. Non-disjunction in der Kette einer gewöhnlichen Monosome sollte also die Ursache der Bildung dieser Form sein.

Dass der letztgenannte Typus keine Asyndese zeigt, kann gewisse Betrachtungen veranlassen. Bei der Erklärung der Asyndese der Nullisomen wurde Gewicht darauf gelegt, dass das Chromosom, das jetzt mit 5.6 bezeichnet wird, fehlte (HÅKANSSON, 1943 a). Es ist aber möglich, dass dieses Chromosom auf die Paarung keinen Einfluss hat, sondern die Asyndese auf die homozygotische Realisierung des Komplexes *g* zurückzuführen sei. Es ist nämlich später ein Zwergtypus mit sehr starker Asyndese gefunden worden, der aus Occidental-Pflanzen mit einer 3-Kette + I hervorgegangen ist und der die homozygotische Realisierung des Komplexes *d* darstellt (HÅKANSSON, 1944 a). Es ist daher nicht unwahrscheinlich, dass auch *g.g* Asyndese zeigen kann. Die abweichende Monosome mit grünem Stengel von Horse Mtn. kann ja als *g.g* + 1 gedeutet werden und zeigte absolute Asyndese. Dies wurde früher auf Nullisomie eines Chromosomenarms zurückgeführt, kann aber durch die Konstitution *g.g* + 1 bedingt werden. Die Monosome mit 5 II + III soll kein Chromosom 5.6 haben, ist aber *g.g* und zeigt keine Asyndese. Hier sind die Forderungen beider alternativen Hypo-

thesen ohne Ergebnis erfüllt. Dies muss auf das Vorkommen eines *a*-Chromosoms aus der Kette beruhen, das die genetischen Bedingungen der Syndese wiederhergestellt hat.

Es scheint mir, dass dieser Einfluss des *a*-Chromosoms verständlicher ist, wenn die Asyndese der Nullisome durch die Kombination *g.g* als durch den Wegfall des Chromosoms 5.6 hervorgerufen wird. Denn dann sind die die Syndese fördernden und störenden Faktoren in derselben Konfiguration wie in homologen Segmenten vorhanden. Die Asyndese könnte z. B. auf einem Deficiency in einem *g*-Chromosom beruhen, dass nur homozygotisch wirksam ist, also bei Gegenwart normaler Allele nicht zutage tritt. Es ist das *a*-Chromosom mit dem dominanten Allel, das das Non-disjunction-Chromosom darstellt.

Kurz sei auf die Eigenschaften der Strukturhomozygoten hingewiesen. *g.g* ist in Santa Rosa 6 verwirklicht; es zeigt keinen sehr ausgeprägten Zwergwuchs, aber sehr ausgeprägte Asyndese. *d.d* ist in Occidental-$zw_1$ realisiert und durch sehr ausgeprägten Zwergwuchs und starke Asyndese gekennzeichnet. Viele *b.b* sind normal, in Corvallis »verkrüppelt» haben wir aber eine mit Zwergwuchs. Asyndese ist aber weder hier noch in einer anderen *b.b* gefunden worden. Dies erinnert an die Verhältnisse bei *Oenothera*, deren »Homozygoten» *b.b* entsprechen. Sie können starken Zwergwuchs zeigen, haben aber keine Asyndese. Möglicherweise deutet die Asyndese der beiden ersten Typen auf ein Deficiency in den *d*- und *g*-Komplexen, das also im *b*-Komplex, der gewöhnliche Ringe verursacht, nicht vorkommt.

Die Frage, warum einige Strukturheterozygoten konstant sind, d. h. warum die beiden »Homozygoten» nicht realisiert werden, soll hier nicht erörtert werden. DARLINGTON (1936) hat aber auf einen zweiten Faktor bei der Konstanz der Heterozygoten hingewiesen, nämlich die Einschränkung des Crossing-over (»limitation of crossing-over»). Wäre das Crossing-over bei den Ringchromosomen normal, dann wäre die konstante Heterozygotie in Frage gestellt. DARLINGTON (1931) äusserte schon früher den Gedanken, dass in der Mitte der *Oenothera*-Chromosomen jederseits des Zentromers ein »differential segment» liegt, dessen Gene sehr stark gekoppelt sind, und in dem kein Crossing-over vorkommt. Dieser Gedanke hat allgemeine Zustimmung erhalten, er wird »durch alle Erfahrung bestätigt» (RENNER, 1942, S. 178). Die vereinten Endsegmente von zwei Nachbarchromosomen sind dagegen einander gleich, und ihre Gene zeigen Crossing-over mit Austauschwerten bis zu 50 % für die Gene *Co—co* für Blütengrösse, die sich also in Kreuzungen so verhalten, als ob sie ihren Locus in einem II anstatt in einem Ring

hätten (Genenkarte s. CATCHESIDE, 1940). Die Gene, die die verschiedenen Genkomplexe charakterisieren, liegen in den differenten Segmenten.

HIORTH hat bei seinen Untersuchungen gefunden, dass *Godetia Whitneyi* dadurch ausgezeichnet ist, dass in mehreren Koppelungsgruppen alle Gene sehr stark gekoppelt sind. Dies gilt z. B. für die Chromosomen 3.4 und 5.6 des Standardkomplexes. HIORTH (1944 a) schlägt vor, »das *F*-Chromosom (Gen für Blütenfleck) mit 1.2 zu bezeichnen, das *C*-Chromosom (Gen für Kotyledonenzeichnung) mit 3.4, das $w_3$-Chromosom mit 5.6. Die beiden ersteren Chromosomen bilden allem Anschein nach den *a*-Teil des 4-Ringes der Monosomen aus Tocaloma». Das Chromosom 5.6 bildet ja das I. Durch diese Eigenschaft der *Whitneyi*-Chromosomen gleichen sie den differenten Segmenten von *Oenothera*, was für die Konstanz gewisser Strukturheterozygoten sicher von Bedeutung ist.

HIORTH (l. c.) hat die Hypothese aufgestellt, dass die Endsegmente der *Whitneyi*-Chromosomen genenfrei sind, während die genenhaltigen Teile sich in der Mitte der Chromosomen befinden sollen. Crossing-over geschieht wohl in den genenfreien Endsegmenten, nicht aber in der Chromosomenmitte. Dies erklärt die starken Koppelungen. In der späten Diakinese und Metaphase 1 sieht man nur terminale oder subterminale Chiasmen, die früheren Stadien entziehen sich aber einer zytologischen Analyse. In stärker entfärbten Präparaten zeigen in der frühen Diakinese die Chromosomen einen dicken, stark gefärbten Teil, in dem sehr wahrscheinlich das Zentromer liegt. Die anderen Teile des Chromosoms sind dünn, schwach gefärbt und zeigen oft die Chromatiden. Ich glaube nicht, dass die besser gefärbten Teile aus Heterochromatin bestehen, wie ähnliche Bilder von *Oenothera* durch gewisse Forscher gedeutet werden. Die stärkere Färbung kann auf grössere Dicke beruhen, die Spiralisierung scheint hier schneller zu verlaufen. DARLINGTON (1933) zeigte, dass bei *Agapanthus umbellatus* »the paired chromosomes show earlier condensation in the neighbourhood of the spindle attachments between pachytene and diakinesis». Andererseits wage ich nicht zu behaupten, dass diese dickeren Teile die differenten Segmente sind.

Mehrere Monosomen haben eine immer offene Konfiguration. BURNHAM (1932) hat durch Studien über die meiotische Prophase gezeigt, dass das Vorkommen einer Kette statt eines Ringes in einer *Zea mays*-Rasse mit reziproker Translokation darauf beruht, dass das eine translozierte Segment sehr klein war. Vielleicht ist in Tocaloma

auch das zweite translozierte Segment relativ klein, denn die Kette hatte die Neigung, sich in zwei (offene) II aufzuteilen. Es fragt sich aber, ob die 4-Konfiguration nicht komplizierter als eine einfache reziproke Translokation ist.

Wie hervorgehoben, sind die Monosomen in *G. Whitneyi* kräftige Typen, und auch die Nullisome ist gut entwickelt. Dies könnte darauf beruhen, dass das Chromosom 5.6 sich doch im Komplex *g* findet, aber nicht als freies Chromosom. Man kann sich mit HIORTH vorstellen, dass die genentragenden Teile des Chromosoms 5.6 in einem der *g*-Chromosomen der Kette vorhanden sind. HIORTH hat verschiedenes angeführt, dass zu Gunsten dieser Auffassung spricht. Wichtig sind die Ergebnisse der Kreuzung monosomischer Toc-$C^{pa}$ mit verschiedenen Rezessiven, die S. 137 referiert wurden. Von $w_3$, *kr* und *sp*, die zur selben Koppelungsgruppe und zum Chromosom 5.6 in den normalen Rassen gehören, zeigt nur $w_3$ Pseudodominanz. *sp* und *kr* können daher nicht im I der Monosome liegen, sie befinden sich sehr wahrscheinlich im *g*-Teil der Kette.

Die zytologischen Beweise dafür, dass Teile des fehlenden Chromosoms sich in der Kette befinden, sind nicht so stark. Vor allem beobachtet man nie eine Paarung des I mit der Kette. Nun kann dies ja darin seine Erklärung finden, dass man keine solche Paarung erwartet, da ein Segment, in dem nach der Hypothese keine Chiasmen zu erwarten sind, nach dem *g*-Chromosom transloziert ist. Nur in der Monosome $C^{st}$ $C^{sp}$ wurde selten eine Anordnung gefunden, die als eine Chiasmabildung zwischen einem *g*-Chromosom und einem Segment .5 oder .6 gedeutet werden kann. Die Grösse des Endchromosoms kann aber darauf hindeuten, dass es ein Stück von einem anderen Chromosom enthält. Es ist das grösste Chromosom des Kerns. Dies kann kaum ein Resultat des primären Austausches zwischen 1.2 und 3.4 sein, denn das zweite *g*-Chromosom der Kette sollte dann klein sein. Dies ist aber nicht der Fall, es ist ebenso gross wie das grössere der *a*-Chromosomen. Zusammen sind die beiden *g*-Chromosomen der Kette bedeutend länger als die *a*-Chromosomen. Andererseits kann das grosse Endchromosom nicht den ganzen Chromosomenkörper von 5.6 besitzen; so gross ist es nicht, es kann sich nur um einen Teil dieses Chromosoms handeln.

Wie soll man sich die Prozesse vorstellen, die dazu führen, dass schliesslich eine *Godetia* mit 12 statt 14 Chromosomen entsteht, eine Form, die doch auf Grund ihrer Asyndese nicht fortbestehen kann? DUBININ (1934) hat aus gewöhnlicher *Drosophila melanogaster* eine

Rasse mit 6 grossen Chromosomen hergestellt. Dies erfolgte durch eine von X-Strahlen hervorgerufene Translokation des aktiven Teils vom kleinen Chromosom IV zum Y-Chromosom. Dieses neue Chromosom zeigt Crossing-over mit dem X-Chromosom. Dadurch entsteht ein komplexes Chromosom mit Teilen von X, Y und IV. Es wurden Weibchen mit zwei solchen Chromosomen sowie Männchen mit einem solchen und einem normalen Y-Chromosom gebildet. Der nach der Translokation übrigbleibende Teil vom Chromosom IV war genetisch inaktiv und ging verloren. STONE und GRIFFIN (1940) haben auch eine Verminderung der Chromosomenzahl von *Drosophila melanogaster* erzielt. Chromosom IV war in diesem Fall aber oft nicht verschwunden. In sehr günstigen Zellen konnten ein oder zwei winzige Mikrochromosomen beobachtet werden, die am ehesten als freie Zentromeren zu bezeichnen waren. Die Veränderungen bestehen auch hier in Translokationen.

In der Gattung *Crepis* sind reziproke Translokationen nach X-Bestrahlung sehr häufig, und die neuen Chromosomen können hier auffallend häufig homozygotisch realisiert werden. GERASSIMOWA (1939) erhielt so von *C. tectorum* eine Pflanze mit einer Translokation zwischen den Chromosomen A und D, eine andere mit einer zwischen B und C. Diese Pflanzen wurden gekreuzt und eine *Crepis nova* I wurde erhalten, »homozygous for all the four translocated chromosome pairs». TOLOGY (1943) hat, wie aus einem Referat in der Zeitschrift Nature hervorgeht, die Chromosomen von *Crepis neglecta* (A, B, C, D) und *fuliginosa* (A, B, D) durch Messungen genau verglichen. Er kommt zur Auffassung, dass die letztere Art von *neglecta* abstammt; die verminderte Chromosomenzahl von *fuliginosa* ist wahrscheinlich durch reziproke Translokation entstanden. Durch eine solche Translokation zwischen B und C in *neglecta* wurde ein grosses Chromosom, das das B-Chromosom von *fuliginosa* darstellt, und ein sehr kleines Chromosom gebildet. Letzteres, das genetisch inert ist, stellt das Zentromer dar und geht bald verloren. Es ist dies eine Hypothese, die sich auf die Untersuchungen von DUBININ stützt.

In ähnlicher Weise kann man sich die strukturellen Veränderungen vorstellen, die zur Bildung des 6-chromosomigen *g*-Komplexes führen. In einer Pflanze, die schon eine reziproke Translokation zwischen den Chromosomen 1.2 und 3.4 hat, entsteht ein neuer Austausch zwischen 5.6 und 2.3 (oder 1.4). Dadurch entsteht ein neues Chromosom 2.3, das die meisten genhaltigen Teile von 5.6 hat. Gleichzeitig wird durch den neuen Austausch ein kleines Chromosom, das beinahe (oder ganz) inert ist, und jedenfalls ohne Schädigung entbehrt werden kann, ge-

bildet. Letzteres wird allmählich eliminiert, das I der Monosomen ist ein gewöhnliches 5.6-Chromosom. Das grössere Chromosom, das also Teile von drei Chromosomen enthält (wie die oben erwähnten *Drosophila*-Chromosomen), bleibt aber in den späteren Generationen erhalten. Man könnte sein Erhalten eigentümlich finden, denn wir schlossen aus dem Verhalten einiger Monosomen, dass es homozygotisch nicht realisiert wird, aus dem Verhalten anderer, dass die homozygotische Realisierung starke Asyndese hervorruft. Schliesslich kennen wir kaum Beispiele für das konstante Vorkommen heteromorpher Autosomenpaare unter Pflanzen, die nicht Bastarde sind.

Die Ursache, dass das komplexe Chromosom erhalten bleibt, liegt darin, dass das Segment von 5.6 nach einem Chromosom transloziert ist, das schon eine reziproke Translokation erfahren hat. Dadurch wird das Segment in einen Chromosomenring eingefügt. Viele Ringe sind bekannt, in denen zwei angrenzende Chromosomen recht verschiedene Grösse haben können. Die starke Terminalisation und dadurch bedingte Zickzackanordnung der Ringchromosomen in *Oenothera* und *Godetia* bedingen, dass ein solches Chromosom nunmehr einem bestimmten Komplex angehört. Bei den Monosomen verursacht die häufige Elimination des I, dass Gonen mit dem neuen Komplex hier in grossem Überschuss auftreten. Später entstehen, wie in Briceland-$C^{st}$, neue Komplikationen, die die Heterozygote konstant machen.

Die Asyndese der Kombination *g.g* kann, wie oben erwähnt wurde, auf ein Deficiency beruhen. Es ist wahrscheinlich, dass das Deficiency im komplexen Chromosom ist, denn bei der zweiten reziproken Translokation zwischen 2.3 und 5.6 kann ein Segment von 2.3 nach dem kleinen Chromosom transloziert werden, das später eliminiert wird. Ohne den Verlust dieses Segments hätte die Nullisome aus Santa Rosa 6 6 II gehabt, womit in der Gattung *Godetia* eine neue Chromosomenzahl entstanden wäre.

## SUMMARY.

Monosomics of *Godetia Whitneyi* are known from five different natural localities: Tocaloma, Marin Co., Cal., Santa Rosa 6, Sonoma Co., Cal., Horse Mtn. and Dyerville, Humboldt Co., Cal., and Briceland, Or. Their genetics has been studied by Dr. GUNNAR HIORTH, of Ås, Norway. It is his material that has been cytologically examined by me.

The monosomics are structural heterozygotes with a chromosome configuration of 4 o + 4 II + I. The 4-configuration is always an open

chain in Tocaloma, S. Rosa 6 and Horse Mtn., a ring or chain in Dyerville and Briceland; its chromosomes are generally arranged zigzagly, so that alternating chromosomes pass to the same pole. Non-disjunction has, however, been observed at times. The univalent is often eliminated; many more gones with 6 than with 7 chromosomes are formed.

One of the complexes of the monosomics is the standard complex *a* in *G. Whitneyi*. The other consists of only 6 chromosomes and is called *g* here. The chain can be denoted by the chromosomes 3.2—2.1—1.4—4.3, the univalent by 5.6. In the chain the two end-chromosomes are of very different size, the central chromosomes are of about the same size. The larger end-chromosome is the largest chromosome of the nucleus and belongs to *g*, the smaller end-chromosome must then belong to *a*. The *g* chromosomes 2.3 + 1.4 are longer than 1.2 + 3.4. As a rule, chromosome 5.6 is absent in *g*.

Monosomics from Tocaloma show a cotyledon marking $C^{pa}$ that has its locus in one of the *g* chromosomes of the chain, the same applying to the marking $C^{st}$ that characterizes monosomics from Briceland.

Of the four gones formed at the meiosis of the monosomics, *a* — 1 is always sterile, *g*, *g* + 1 and *a* are fertile both in embryo-sacs and pollen, *g* is in great excess in consequence of the elimination of the I. Of the functioning ♀-gones in a cross of the type $C^{pa} \times cc$, 86 % were *g*, 7 % *a* and 6 % *g* + 1. Hence *g* is fertile with or without chromosome 5.6, whereas this chromosome is necessary for the *a* complex.

On being crossed with *cc* and on self-fertilization Toc-$C^{pa}$ gave *a.g* (monosomic $C^{pa}$), *a.g* + 1 (disomic $C^{pa}$) and *a.a* (normal type, *cc*). The disomic $C^{pa}$ has a 4-chain + 5 II and has not any excess of $C^{pa}$ in the progeny. The cross $C^{pa}\ c\ W_3 \times cc\ w_3w_3$ has given 18 $C^{pa}\ W_3$ (4-chain + 5 II) : 264 $C^{pa}\ w_3$ (4-chain + 4 II + I) : 23 $c\ W_3$ (7 II) : 1 $c\ w_3$ (4-chain + 4 II + I). Thus $W_3$ has its locus in the I of the monosomic; $c\ w_3$ must have arisen as a result of crossing-over in the chain. The cross $C^{pa}\ C^s \times cc$ gives rise to some plants with two cotyledon markings, $C^{pa}\ C^s\ c$. They have 14 chromosomes with 5-chain + 4 II + I or 3-chain + 5 II + I, and have arisen by non-disjunction, one of the *a* chromosomes (with $C^s$) of the chain having accompanied the *g* chromosomes.

Monosomics from Santa Rosa 6 split off a large number of nullisomics having the formula *g.g*, which show asyndesis. *a.g* + 1 (4-chain + 5 II) and *a.a* are formed in small numbers. A monosomic

with the configuration 5 II + III was discovered here. It was probably formed by an *a* chromosome in the chain accompanying the *g* chromosomes. Its probable formula is *g.g.* + an *a* chromosome from the chain; the asyndesis produced by the combination *g.g* would be prevented by the presence of the normal allele in this *a* chromosome.

Monosomics from Horse Mtn. split off a small number of plants with 13 chromosomes and complete asyndesis. Probably they are the combination *g.g* + 1. In addition *a.a*, *a.g* and *a.g* + 1 are formed.

Briceland-$C^{st}$ is a constant monosomic. Neither *a.g* + 1 nor *a.a* are split off here. The cross $C^{st} \times cc$ gave only $C^{st}$; amongst 1,108 plants there are only two malformed *cc*, which are probably trisomics. 14-chromosomal *cc* embryos evidently die off here. Other complications exist.

$C^{sp}$ is another cotyledon marking in Briceland. $C^{sp}$ plants have 6 ₀ + 4 II and are denoted as *a.b*. There are also $C^{sp}$ $C^{sp}$ with 7 II (*b.b*). Plants with two markings, $C^{st}$ $C^{sp}$, that is *b.g*, have been obtained by crossing. They are monosomics and usually have 3-chain + 5 II, one II being very heteromorphic. 5-chain + 4 II is rarely formed. The significance of these configurations is discussed (page 148).

In *G. Whitneyi* there are four different kinds of complexes. *a* is the standard complex, the *b* complex produces rings with *a*, the *d* complex yields 3-chain + I with *a*, *g* is a 6-chromosomal complex that produces the 4-configuration + I. There are constant heterozygotes of types *a.b*, *a.d* and *a.g*. Several cytogenetical facts suggest that large portions of chromosome 5.6, which normally does not enter the *g* complex as a free chromosome, are present in the large *g* chromosome in the chain.

It is probable that a reciprocal translocation has taken place between chromosome 5.6 and an already altered chromosome 2.3. This results in the formation of a large complex chromosome with segments from three standard chromosomes and a small remnant chromosome from 5.6, which is lost. Thanks to its coming into a ring the large chromosome is preserved, and will enter a complex *g*. Either the complex chromosome cannot be realized in a homozygotic condition or, if it can be, it gives rise to a strong asyndesis. This asyndesis is suggestive of a deficiency, which may have arisen at the second reciprocal translocation.

Lund, April, 1944.

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# INTERSPECIFIC HYBRIDIZATION WITHIN THE GENUS BETULA

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THE taxonomical literature contains descriptions of rather many spontaneous species hybrids within the genus *Betula* (RHEDER, 1940). For some of these the hybridogenous constitution has been confirmed by pollen examinations (ROSENDAHL, 1916) and meiotic studies (HELMS and JØRGENSEN, 1927; WOODWORTH, 1929, 1930; and JOHNSSON, 1944). WOODWORTH (1931) carried out extensive experiments in species crossing, and a large number of his combinations resulted in »seeds with embryos». These seeds, however, had not yet germinated at the time his report was printed, and the later results of his experiments do not seem to have been published. More advanced experiments have been published by SMITH and NICHOLS (1941), who enumerate nine different combinations, carried out during 1938—39, that resulted in trees whose hybridity has been preliminarily established.

The present paper deals with a number of experiments in species crossing commenced during the years 1941—43. The main purpose of these experiments is to study the fertility of the species crosses and the rate of development of the $F_1$ individuals with regard to their utility in practical forestry (JOHNSSON, 1941).

## METHODS AND MATERIAL.

High-growing lignoses offer, of course, a very unwieldy experimental material. Usually the trees do not fructify until they have reached a rather high age, by which time they are already of considerable size. This makes it an exacting task to carry out crossing experiments in their natural localities. For *Populus* and *Salix*, which are characterized by short seed-ripening time, it has proved that seed-ripening can be forced by water culture of branches in the greenhouse. According to SMITH and NICHOLS (1941), this method seems to have been first used by YANCHEVSKY in 1904 for *Salix* and later by WETTSTEIN (1933) for *Populus*. At the Ekebo institute the method has been employed since 1938, preferably for *Populus* (JOHNSSON, 1940). It has

been found especially successful for species belonging to the section *Leuce*, these species having a specially short seed-ripening time; for species belonging to other sections, which have a longer seed-maturing time, the method has been found to be rather unreliable. The same experience has been had by SMITH and NICHOLS (1941).

As the *Betula* species have a substantially longer seed-ripening time than *Salix* and *Populus*, it was *à priori* less probable that the crossing experiments could be carried out in this genus with the aid of branches in water culture. This *à priori* view was, in fact, corroborated by a rather extensive experiment conducted in the spring of 1939.

In this experiment, which was carried out in a greenhouse provided with electric irradiation, many different nutrient solutions were used. In the best series of experiments the branches kept alive for two months and developed rather large catkins. The seed, however, was not germinable.

Since the method of growing severed branches in water culture was obviously not suitable for birches, experiments were undertaken the following spring to keep the branches alive until the seed had time to mature by grafting them on 2—3 year birch plants inserted in pots.

As a suitable method for carrying out these grafts Mr. H. JENSEN, Director of the Ramlösa Nurseries, recommended »bottle grafting». The procedure is briefly as follows: a branch up to 6—7 dm long with flower buds from the chosen mother-tree is grafted on the side of the stock by means of slits 1—2 dm above the severed end of the graft, the free end of the graft being put in a glass of water. The grafting technique is described in detail by JENSEN (1942). BLAKESLEE and FARNHAM (1923) recommend the method for keeping exceptional breeding material alive. WINKLER (1924) calls the grafting method duplication and states that it was already described and illustrated by MARCO BURSATUS in his »Giardino di Agricultura» (Venice, 1592).

After the cut surfaces of the grafting-point have united (about 2 months later), the free end of the graft-branch is cut away and the result is a small, easily handled dwarf tree, which immediately flowers profusely and can be used as a mother-plant in the crossing experiments. Fig. 1 shows such mother-trees with ripening catkins about three months after the grafting; *a* represents *Betula lutea* and *b Betula verrucosa* (a *pendula* form). The pollen is collected from the cut-off branches of the chosen father-trees. This method is excellent for broad-leaved trees possessing a large seed production. The graft individual in Fig. 1 *b* has over 40 catkins. In variety crosses about 200 plants from each

catkin may be obtained if the culture conditions are good, i. e. over 8.000 plants in the present case. The graft individual survives to the following year and even then often flowers profusely, thus enabling the same clonal plants to be used for the reproduction of an earlier cross or in other crossing combinations for several years.

The crosses are carried out in a greenhouse in the middle of March. In May the mother-trees are moved into the open, and the fruits ripen in June—August.

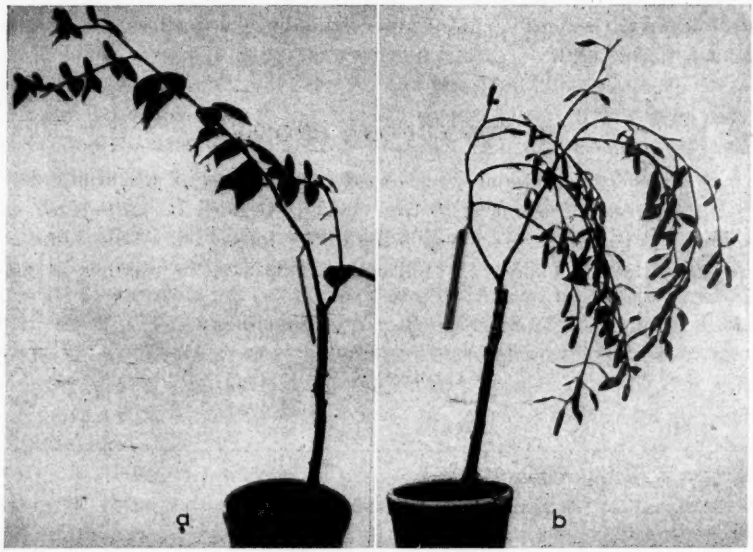


Fig. 1. Bottle grafts, about 3 months after grafting, with ripening catkins; *a* = *B. lutea*, *b* = *B. verrucosa*.

In *Betula*, fruits develop even if the seed has aborted or if pollination has not taken place (MORGENTHALER, 1915; JOHNSSON, 1941). The fruits from one catkin of each cross are therefore examined immediately after the harvest. They are left in water for 24 hours, during which they swell a little. Under a dissecting-microscope with a magnification of × 10 it is then easy to decide whether the fruit contains seed or not. By this means the percentage of seed-containing fruits is determined (Tables 1—3). As a rule, fruits containing seed are 100 % germinable. The bulk of the fruits are stored dry until February the next year, when they are sown in the greenhouse. Fruits with a high percentage of

seeds (as a rule variety hybrids) are sown direct, while fruits with a low percentage of seeds (usually species hybrids) are first inspected under the microscope as described above and assorted, after which only fruits containing seed are sown. After one transplantation in the house the plants are planted out into cold frames and can attain a height of up to one metre the first autumn.

The trees entering as partners in the crosses from indigenous Swedish species have been selected from various localities in Sweden. Material from foreign species have been taken from trees cultivated in the Botanical Gardens of Lund and Gothenburg as well as the Bergian Garden in Stockholm.

## B. VERRUCOSA CROSSES.

*B. verrucosa*, a diploid species with 2n=28 (HELMS and JØRGENSEN, 1927), has been crossed with five species (besides *B. pubescens*, see below). One of the crosses was in both directions. The results obtained are submitted in Table 1. This contains data as to number of ripe

TABLE 1. *B. verrucosa crosses.*

| Mother | | Father | | No. of ripe catkins | No. of fruits per catkin | % fruits containing seed | No. of $F_1$ plants | 2n of $F_1$ | Year |
|---|---|---|---|---|---|---|---|---|---|
| Species | Tree No. | Species | Tree No. | | | | | | |
| *B. verrucosa* EHRH. ..... | 13 | *B. papyrifera* MARSH. ..... | 319 | 46 | 497 | 8,8 | 1000 | 56 | 1941 |
| *B. verrucosa* EHRH. ..... | 13 | *B. Ermani* CHAM. | 92 | 11 | 576 | 0,9 | 12 | 42 | 1941 |
| *B. verrucosa* EHRH. ..... | 13 | *B.* sp. ..... | 87 | 43 | 539 | 1,7 | — | — | 1943 |
| *B. verrucosa* EHRH. ..... | 110 | *B. Maximowiczziana* REG. ..... | — | 68 | 277 | 0,4 | 8 | 28 | 1942 |
| *B. verrucosa* EHRH. ..... | 13 | *B. japonica* WINKL. ..... | 100 | 39 | 389 | 53,2 | 750 | 28 | 1941 |
| *B. japonica* WINKL. ..... | 100 | *B. verrucosa* EHRH..... | — | 8 | 279 | 28,3 | 475 | — | 1942 |

catkins, number of fruits per catkin, percentage of fruits containing seed, number of $F_1$ plants, and the chromosome number of the $F_1$ for each cross carried out; in addition, the year of crossing is stated. The percentage of »fruits containing seed» is of course an expression of the fertility of the cross in question. By way of comparison it may be stated

that 11 variety crosses within *B. verrucosa* have yielded on an average 52,3 % fruits with seed, with a variance from 4,7 to 87,0 %. All species crosses, possibly except one, show reduced fertility. The highest fertility is shown by the cross *B. verrucosa* × *B. japonica* with 53,2 % fruits containing seed. Both the parents are diploid in this cross and belong to the same section of the genus *Betula*, viz. sect. *Albae*. Morphologically the species are very close to each other, but they have widely separated areas of distribution (SCHMUCHER, 1942), *B. verrucosa* is European and *B. japonica* is East-Asiatic. The $F_1$ population, now two years old and comprising 750 plants, shows a very marked heterosis (see below); otherwise it agrees morphologically closely with *B. verrucosa* in leaf-shape and wartiness of the shoots. The reciprocal cross, now one year old and numbering 475 plants, shows a lower fertility, and, moreover, the heterosis effect does not seem to be so pronounced. However, these observations are not certain.

A surprisingly good fertility was shown by the cross *B. verrucosa* × *B. papyrifera*, with 8 % seed-containing fruits. The *B. papyrifera* tree entering as partner into it belongs to var. *occidentalis* HOOK, which has $2n = 84$ (WOODWORTH, 1930), this being confirmed by the 2n-number, 56, of the $F_1$. The two-years-old $F_1$ population, which comprises 1.000 plants, shows a very distinct heterosis. Morphologically it stands considerably nearer the father than the mother species in respect of size and shape of leaves. The shoots of most of the plants are glandular.

An exceedingly low fertility is shown by the combination *B. verrucosa* × *B. Ermani*. Direct determinations of the chromosome number of the father species are not available. Since the $F_1$ has $2n = 42$, however, *B. Ermani* must have $2n = 56$. Hence there is a difference in chromosome number between the parents; further, *B. Ermani* belongs to sect. *Costatae*, from which the low fertility of the cross is well explained. Still lower fertility is shown by the hybrid *B. verrucosa* × *B. Maximowicziana*. Here, too, the parent species belong to different sections, *B. Maximowicziana* being classed under sect. *Acuminatae*. Both species are however diploid, and the $F_1$ has $2n = 28$. The merely one-year-old $F_1$ plants are too small for determination of the hybridity on morphological grounds.

The species of the father-tree in the cross *B. verrucosa* × *B.* sp. has not admitted of being determined. The tree which is cultivated in the Lund Botanical Garden is labelled *B. grossa*. This species, however, belongs to sect. *Costatae*, and the tree in question belongs in any case to

sect. *Albae*. In all probability it is a form of *B. papyrifera* or a species closely related to this. The seed has not yet been sown.

## B. PUBESCENS CROSSES.

The tetraploid species *B. pubescens* enters as mother in six different species combinations — as well as in combination with *B. verrucosa* (see below). Most of the combinations have been made with different *B. pubescens* individuals as mother. The results are brought together in

TABLE 2. *B. pubescens crosses.*

| Mother | | Father | | No. of ripe catkins | No. of fruits per catkin | % fruits containing seed | No. of $F_1$ plants | 2n of $F_1$ | Year |
|---|---|---|---|---|---|---|---|---|---|
| Species | Tree No. | Species | Tree No. | | | | | | |
| *B. pubescens* EHRH. | 1 | *B. papyrifera* MARSH. | 319 | 12 | 146 | 1,4 | 40 | 70 | 1942 |
| *B. pubescens* EHRH. | 1 | *B. papyrifera* MARSH. | 319 | 105 | 286 | 1,8 | — | — | 1943 |
| *B. pubescens* EHRH. | 5 | *B. papyrifera* MARSH. | 319 | 18 | 523 | 4,6 | 200 | 70 | 1941 |
| *B. pubescens* EHRH. | 5 | *B. papyrifera* MARSH. | 319 | 15 | 352 | 30,4 | — | — | 1943 |
| *B. pubescens* EHRH. | 1 | *B. Ermani* CHAM. | 92 | 10 | 101 | 9,9 | 18 | 56 | 1941 |
| *B. pubescens* EHRH. | 5 | » | 92 | 1 | 293 | 0,0 | — | — | 1941 |
| *B. pubescens* EHRH. | 120 | *B.* sp. | 87 | 8 | 312 | 54,5 | — | — | 1943 |
| *B. pubescens* EHRH. | 125 | » | 87 | 31 | 306 | 59,5 | — | — | 1943 |
| *B. pubescens* EHRH. | 1 | *B. albo-sinensis* BURK. | — | 7 | 209 | 2,4 | 30 | 56 | 1942 |
| *B. pubescens* EHRH. | 132 | *B. albo-sinensis* BURK. | — | 12 | 251 | 0,0 | — | — | 1943 |
| *B. pubescens* EHRH. | 136 | *B. albo-sinensis* BURK. | — | 3 | 268 | 0,0 | — | — | 1943 |
| *B. pubescens* EHRH. | 1 | *B. lenta* L. | | 5 | 205 | 3,4 | 26 | 56 | 1942 |
| *B. pubescens* EHRH. | 5 | » | | 8 | 208 | 0,0 | — | — | 1943 |
| *B. pubescens* EHRH. | 128 | » | | 24 | 304 | 0,0 | — | — | 1943 |
| *B. pubescens* EHRH. | — | *B. lutea* MICHX. | | 8 | 243 | 0,0 | 10 | 56 | 1942 |

Table 2. For 14 variety crosses within the species the percentage of »fruits containing seed» has been 62,1, varying between 19,5 % and 87,7 %. The cross *B. pubescens* × *B. papyrifera* has been carried out with two different *B. pubescens* individuals, and the same crosses have been repeated for two years. The cross with tree No. 1 as mother has shown low fertility, viz. respectively 1,4 and 1,8 % fruits with seed in the years 1942 and 1943. With tree No. 5 as mother, the fertility has been higher but has varied greatly, with 4,6 % seed-containing fruits in 1941, and not less than 30,4 % in 1943. From this it is clearly seen that the result of the cross depends partly on the individuals used, partly on external conditions. The environmental circumstances needing primary attention are no doubt those connected with the pollination. The stigmata of different flowers in the same catkin are not simultaneously conceptible, and moreover there is a time-difference between the different catkins. In the crossing work all the catkins have therefore, as a rule, been pollinated every morning three days in succession. Still, it is possible that more stigmata have been conceptible on one crossing occasion than on another. Further, the quantity of pollen used, which to a certain extent has depended on the supply of pollen available at the moment, may exercise some influence. Pollen for the pollinations has usually been stored some days (in an exsiccator over chloride of lime), and there is therefore a possibility that the state of freshness of the pollen may influence the result of the cross. As expected, $F_1$ has $2n = 70$. The two-year-old plants from the 1941 cross show closer agreement in leaf-size and leaf-shape with the father than with the mother species. The heterosis effect is very pronounced (see below).

Of the two crosses carried out in the combination *B. pubescens* × *B. Ermani*, only the one gave fruits with seed, from which a few plants have been obtained. These are however completely matroclinous and are probably attributable to the ingress of stray pollen from *B. pubescens*, a possibility that is unfortunately not entirely excluded, as pollen from this species had been used in the same greenhouse. In any case the cross combination is highly sterile, which agrees with the taxonomic assignment of the parents to different sections.

The two crosses carried out between *B. pubescens* and the previously mentioned birch of undetermined species, which nevertheless is probably a *B. papyrifera* form, have both proved very fertile. The percentages of fruits containing seed are respectively 54,5 and 59,5. This high fertility makes it probably that the undetermined species has $2n = 56$. However, neither the chromosome number of the tree used nor that of the

$F_1$ has yet been determined. Within *B. papyrifera* 56-chromosomal forms occur (WOODWORTH, 1929). The cross *B. pubescens* × *B. albo-sinensis* has been carried out with three different *B. pubescens* trees as mothers. One cross alone has given a few plants. The leaf-shape of these is distinctly intermediate, and therefore these plants are undoubtedly $F_1$ individuals. The 2n-number of the $F_1$ is 56, and therefore *B. albo-sinensis* must also be assumed to have this 2n-number. It belongs to sect. *Costatae*.

The *B. pubescens* × *B. lenta* cross carried out with three different mother-trees has only given progeny in one case. This is, however, completely matroclinous and has $2n = 56$ as against 42, which was to be expected in the $F_1$, since the chromosome number of *B. lenta* is $2n = 28$ (WOODWORTH, 1929). Hence the combination must in any case be very sterile.

The catkin examined from the cross *B. pubescens* × *lutea* possessed only fruits without seed. From the other seven catkins, however, ten plants were obtained, but these are all entirely matroclinous. Their 2n-number is also 56 as against 70, which is according to expectation, since *B. lutea* is hexaploid with $2n = 84$ (WOODWORTH, 1929). Consequently, this cross is also extremely sterile.

## B. PUBESCENS × B. VERRUCOSA AND RECIPROCAL.

In Table 3 the results are gathered from a number of crosses between the two European species, the 28-chromosome *B. verrucosa* and the 56-chromosome *B. pubescens*. It will be seen that of six crosses, three in each direction, only two plants have been obtained. These have the 2n-number, 42, expected for the $F_1$. These crosses comprise altogether 73 catkins with about 17.000 florets. The combination must therefore be regarded as extremely sterile. A seventh cross in the direction *B. pubescens* × *B. verrucosa* has yielded ten plants, all of which however have the 2n-number, 56, of the mother species. Probably these plants have arisen through the intrusion of *B. pubescens* pollen. Hypothetically, of course, there are also two other possibilities: (1) pseudogamy may conceivably be present, (2) *B. verrucosa* may, in very low frequency, form unreduced pollen-grains, which may have functioned in this cross. That *B. verrucosa* may produce unreduced gametes is evident from the fact that autotriploid *B. verrucosa* individuals have been met with in a couple of cases in nature (JOHNSSON, 1944).

TABLE 3. *B. pubescens* × *B. verrucosa and reciprocal.*

| Mother | | Father | | No. of ripe catkins | No. of fruits per catkin | % fruits containing seed | No. of $F_1$ plants | 2n of $F_1$ | Year |
|---|---|---|---|---|---|---|---|---|---|
| Species | Tree No. | Species | Tree No. | | | | | | |
| *B. verrucosa* EHRH. | 13 | *B. pubescens* EHRH. | 80 | 6 | 399 | 1,5 | 2 | 42 | 1941 |
| *B. verrucosa* EHRH. | 13 | *B. pubescens* EHRH. | 75 | 1 | 542 | 0,2 | 0 | — | 1941 |
| *B. verrucosa* EHRH. | 24 | *B. pubescens* EHRH. | 130 | 20 | 183 | 0,0 | — | — | 1942 |
| *B. pubescens* EHRH. | 1 | *B. verrucosa* EHRH. | 266 | 38 | 202 | 0,0 | — | — | 1943 |
| *B. pubescens* EHRH. | 135 | *B. verrucosa* EHRH. | 266 | 8 | 322 | 0,3 | — | — | 1943 |
| *B. pubescens* EHRH. | 118 | *B. verrucosa* EHRH. | 269 | 3 | 210 | 0,0 | — | — | 1943 |
| *B. pubescens* EHRH. | 5 | *B. verrucosa* EHRH. | — | 11 | 282 | 0,5 | 10 | 56 | 1942 |

## THE HETEROSIS EFFECT IN SOME CROSSES.

In order to make a close study of the rate of growth and other characters possessed by the hybrids in comparison with the parental species, comparative variety tests are being started as soon as the plants become sufficiently large. As a rule, these tests are being arranged according to FISHER's block method (FISHER, 1938), the different plots being planted in two rows with 25 trees in each row, and, as a rule, in triplication (JOHNSSON, 1939). The trials so far planted out comprise the three $F_1$ populations produced in the year 1941, viz. *B. pubescens* × *B. papyrifera*, *B. verrucosa* × *B. papyrifera* and *B. verrucosa* × *B. japonica*. There is further included a family, constituted of plants from seed from a tree after open pollination from each of the species *B. verrucosa*, *B. pubescens* and *B. papyrifera*. The tests were started in the spring of 1943. Unfortunately the plants of the different varieties were, nursery-fashion, not of fully equal value on being planted out. The $F_1$ populations consisted of one-year-old transplanted plants. The two species *B. verrucosa* and *B. pubescens* were non-transplanted two-years-old plants, and, finally, *B. papyrifera* consisted of transplanted two-years-old plants. This lack of uniformity is probably levelled out after a couple of years, but in any case it sets its mark on the growth results of the first year. *B. papyrifera* is especially favoured by the

higher age the transplanted plants had on being planted out. In spite of this, the growth shows even the first year after planting out distinctly significant differences, as can be seen from the following results of measurements:

| | Growth height in cm | Relative index |
|---|---|---|
| *B. pubescens* × *B. papyrifera* | 70,2 | 177 |
| *B. verrucosa* × *B. papyrifera* | 73,4 | 185 |
| *B. verrucosa* × *B. japonica* | 67,3 | 170 |
| *B. verrucosa* | 39,6 | 100 |
| *B. pubescens* | 50,2 | 127 |
| *B. papyrifera* | 79,2 | 200 |

An analysis of the variance of the test gives the following result:

| | Degrees of Freedom | Sum of Squares | Mean Square | Standard Deviation |
|---|---|---|---|---|
| Blocks | 2 | 851,70 | 425,85 | |
| Varieties | 5 | 3.455,28 | 691,06 | 83,13 |
| Error | 10 | 197,11 | 19,71 | 4,44 |
| Total | 17 | 4.504,09 | | |

This gives a mean experimental error of $4{,}44/\sqrt{3} = 2{,}56$ cm. The difference in growth rate between the $F_1$ populations and the species *B. verrucosa* and *B. pubescens* is thus placed beyond all doubt. The differences between the $F_1$ populations among themselves are, however, not significant. The barely significant superiority that *B. papyrifera* shows over the hybrids is doubtless entirely due to the fact that the *B. papyrifera* plants were older on being planted out.

## DISCUSSION.

The experimental results set forth above show that in a number of combinations the *Betula* species are interfertile to a rather high degree. Especially does this seem to apply to combinations between species belonging to the same section, at any rate respecting the section *Albae*. On the other hand, species belonging to the section *Albae* are extremely, if not absolutely, sterile in combinations with species of the section *Costatae*. The section limit thus seems to represent a very effective sterility barrier. An at least equally effective sterility barrier separates,

however, the two *Albae* species *B. verrucosa* and *B. pubescens*. Between these two species it has been found very hard to obtain even single hybrids. This circumstance is of special interest in view of the fact that intermediate forms between these two species, which have largely the same area of distribution, are very numerous in nature. For a long time these intermediate forms have been regarded by taxonomists as hybrids. For instance, as early as 1865 REGEL writes: »*B. pubescens* EHRH. und *B. verrucosa* EHRH., sind als zwei . . . vollständig in einander übergeführte Arten anzusehen». MORGENTHALER (1915) arives at the same result on the basis of statistical analyses of the variation. He states: »*B. alba* L. besteht aus zwei extremen Sippen, *B. verrucosa* und *B. pubescens*, und zahlreichen, durch Kreuzung derselben entstandenen Mischformen». HELMS and JØRGENSEN (1927) found that *B. verrucosa* is diploid with $2n = 28$ and *B. pubescens* tetraploid with $2n = 56$. They also describe a 42-chromosomal tree, which was interpreted by them as a $F_1$ between the two species. This provided a diagnostic aid for the determination of the hybrid frequency in *B. alba* populations. Extensive investigations of natural populations, however, have shown that individuals with triploid and aneuploid 2n-numbers are very rare (JOHNSSON, 1941; WETTSTEIN and PROPACH, 1939). Furthermore, it has proved that the few spontaneous, probable $F_1$ individuals are absolutely female-sterile (HELMS and JØRGENSEN, 1927; JOHNSSON, 1944).

It seems therefore but little probable that 42-chromosomal hybrids can play a noteworthy part in the origin of intermediate forms between the two species. One hypothetical possibility remains however, viz. that the intermediate forms may be derived from 56-chromosomal hybrids that have arisen from unreduced gametes from *B. verrucosa*. The fact that *B. verrucosa* actually produces unreduced and functional gametes is proved by the existence of rare spontaneous autotriploid individuals (JOHNSSON, 1944). Among the *B. pubescens* × *verrucosa* crosses contained in Table 3, one is noticed that has given ten 56-chromosomal plants. Although these are probably to be attributed to experimental errors, there is the possibility that they have arisen from unreduced *B. verrucosa* gametes. However, further investigations are necessary here. No doubt the most probable explanation of the intermediate forms is that *B. pubescens* is a highly variable species that includes a great many *B. verrucosa* characters within its range of variation. The fact is that the intermediate forms seem always to have the chromosome number 56 (JOHNSSON, 1941) — disregarding the rare 42-chromosomal individual.

The sterility barrier that separates *B. verrucosa* and *B. pubescens* cannot be solely explained by the difference in chromosome number, as the crosses between these two species and the 84-chromosomal *occidentalis* form of *B. papyrifera* are relatively fertile. $F_1$ populations of 1.000 plants have been produced without difficulty. This high interfertility must be considered to indicate that the North-American *B. papyrifera* is fairly closely related to the European *B. pubescens* and *B. verrucosa*. In fact, REGEL (1865) gives all three species as subspecies of *B. alba* L. and regards *B. papyrifera* as the North-American form of *B. pubescens*. The same view was advanced anew by FERNALD (1902).

The high fertility of the hybrid between the two diploid species *B. verrucosa* and *B. japonica* belonging to the section *Albae* is in the best agreement with the obvious morphological resemblances of these species.

SMITH and NICHOLS (1941) state — without giving any particulars — that apparent hybrid plants have been obtained from the following interspecific crosses within the sect. *Albae*: *B. japonica* × *populifolia* and *B. papyrifera*, *B. papyrifera* var. *kenaica* × *B. japonica*, and *B. populifolia* × *B. papyrifera*.

A spontaneous hybrid belonging here is the *B. coreulea-grandis* × *populifolia* described by WOODWORTH (1929).

The tested interspecific combinations between species belonging to sect. *Albae* and species belonging to sect. *Costatae* have all turned out highly sterile. Only in the combinations *B. verrucosa* × *B. Ermani* and *B. pubescens* × *B. albo-sinensis* have a few $F_1$ plants been obtained with certainty. NICHOLS and SMITH (1941) mention three crosses of this type: *B. japonica* × *B. lenta* and × *B. nigra* as well as *B. populifolia* × *B. lenta*.

The cross *B. verrucosa* × *B. Maximowicziana*, sect. *Acuminatae*, has given a few plants (see Table 1), which are however as yet too small to determine whether they are actually $F_1$. NICHOLS and SMITH state, however, that the cross *B. japonica* × *Maximowicziana* has been successful.

No attempts have been made with species belonging to sect. *Nanae*. However, unmistakable spontaneous hybrid individuals are met with in Sweden between *B. nana* and the two *Albae* species *B. verrucosa* and *B. pubescens*. Spontaneous hybrids between *B. glandulifera*, on one hand, and *B. papyrifera* and *B. lutea*, on the other hand, are described by ROSENDAHL (1916) and WOODWORTH (1929, 1930), who also mention *B. lenta* × *B. pumila*.

Thus, it is evident that interspecific hybridization within the genus *Betula* is also possible to a wide extent between species belonging to different sections. The fertility of the species hybrids, however, is variable. Especially in crosses between sections the fertility is low in all known cases. In crosses between species within the same section, in any case respecting the section *Albae*, it has proved that a number of cross combinations possess a fertility fully sufficient for practical purposes. It has also been found that certain $F_1$ populations exhibit a marked heterosis effect.

## SUMMARY.

(1) A method that greatly facilitates the carrying out of crossings between tall lignoses is described.

(2) Interspecific crossing experiments between *B. verrucosa* and other species are reported. Good fertility is possessed by the combination *B. verrucosa* × *B. papyrifera* and especially *B. verrucosa* × *B. japonica*. The hybrids are luxuriant.

(3) Interspecific crossing experiments between *B. pubescens* and other species show rather good fertility and heterosis in the combination *B. pubescens* × *B. papyrifera*.

(4) Crosses between species belonging to different sections are highly sterile.

(5) The cross *B. pubescens* × *B. verrucosa* and reciprocal is extremely sterile.

(6) The results obtained are discussed, special stress being laid on the lack of agreement between the extreme sterility in the cross *B. verrucosa* × *B. pubescens* and the general conception that the numerous intermediate forms in Nature between these species are hybridogenous in origin.

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# CYTOLOGICAL STUDIES IN TRITON

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## INTRODUCTION.

THIS investigation is concerned with changes in the chromosome number of the salamander *Triton taeniatus,* spontaneous or artificially produced. The emphasis is laid on the occurrence of triploidy and its cytological consequences.

Being very common in the plant kingdom, it is only during recent years that cases of polyploidy have been noticed in the animal kingdom. The central position of polyploidy in plant genetics has been clearly shown by MÜNTZING (1936) and others. Quantitative changes in the mass of chromosomes and genes must have played a very important part in the development of plants towards greater variability and with it more appropriate adaptations to the demands of their environment. Obviously, circumstances within the animal kingdom are in sharp contrast to this. Here polyploidy seems to be a relatively rare phenomenon. Caution must be used in making definite statements as to its frequency and possible significance, because hitherto the animal series have not been systematic objects of cytological research. The chromosome numbers are known only for a small number of described species. A summary has recently been made by MCCLUNG (1939).

As regards the lower vertebrates (*Pisces, Amphibia* and *Reptilia*), which are especially interesting in this connection, exact chromosome numbers are known for about 170 species. In about 40 % these numbers are multiples of 12 (24, 36, 48, 60 and 84). The numbers 24 and 36 are the most common and are found in 34 and 28 species respectively.

VANDEL (1937) has summarized the known cases of polyploidy in animals. A few of them belong to the invertebrates (mainly *Arthropodians*) and 3 cases to the vertebrates (*Amphibians*). As regards the vertebrates, the occurrence of polyploidy (and haploidy) is as yet only known in the class *Amphibia.* The following is a list of the cases hitherto published. x indicates the basic number.

(1) *Rana esculenta* (3x). G. and P. HERTWIG (1920). Spontaneous.
(2) *Rana fusca* (3x). DALCQ (1930). Spontaneous.
(3) *Rana pipiens* (3x). PARMENTER (1933). Parthenogenetic.

(4) *Triton palmatus* (3x). FANKHAUSER (1934). Experimental?

(5) *Triturus viridescens* (3x). FANKHAUSER (1938 a). Spontaneous (and experimental?).

(6) *Triturus viridescens* (3x and x). FANKHAUSER and GRIFFITHS (1939), GRIFFITHS (1941). Experimental.

(7) *Eurycea bislineata* (3x and 4x). FANKHAUSER (1939 a). Spontaneous.

(8) *Triton taeniatus* (x). BALTZER (1922). Experimental.

(9) *Triton taeniatus* (3x and x). BÖÖK (1940, 1941, 1943). Spontaneous and experimental.

(10) *Triturus viridescens* (5x). FANKHAUSER (1940). Spontaneous.

In cases (1) to (4) are included triploid embryos which were accidentally found during other investigations (cf. FANKHAUSER's summary, 1938 a). The other cases, (5) to (10), are represented by cases of polyploidy or haploidy of spontaneous origin or which have been experimentally induced. So far only one adult triploid individual has been found in a natural population. A preliminary note on this has been communicated earlier (BÖÖK, 1940). This animal, which was a *Triton taeniatus* ♂, also offered possibilities for studying meiosis, which will be described in a later chapter. The other cases are all concerned with larvae.

The most important object of the investigations on polyploid animals of sporadic appearance in nature or produced by experiments seems to be the confirmation or non-confirmation of general cytological laws. It seems, however, less important in the present initial stage of zoological polyploidy research to make detailed comparisons with botanical material. The main question is thus clear: Is the addition of further genomes in animals accompanied by the same gross morphological changes as in plants, i.e. an increased body size, and by the same histological, cytological and cytogenetical changes? A short review follows of the results so far obtained on this subject.

## NATURAL POLYPLOIDY.

MULLER (1925) was led to the following reflections by the contrast between the frequency of polyploids in animals and plants respectively. The difference is no doubt due to the fact that the majority of polyploid plants are hermaphrodites, while most animals are bisexual. In the case of animals this implies that polyploidy would produce a

balance of chromosomes which is disharmonious for sex determination. This should give sexually abnormal or sterile types. The idea is chiefly based upon BRIDGES's (1922) well-known investigations of *Drosophila* regarding the significance of the balance between X-chromosomes and autosomes for sex determination. Here polyploidy leads to the development of intersexes and other complications that further endanger sexual reproduction. If this is valid for other animals, too, the only way to avoid sexual disturbances in connection with polyploidy would be by parthenogenetic reproduction. It is therefore of interest that among animals cases are known in which polyploidy is connected with parthenogenetic breeding.

VANDEL (1928, 1934) investigated two races of the species *Trichoniscus elisabethae*. The one proved to be bisexual with the normal proportion between ♀♀ and ♂♂, i. e. 1 : 1. The other consisted almost exclusively of ♀♀ (100 ♀♀ : 1,6 ♂♂). The cytological examination showed that the bisexual race was diploid (2x = 16), while the parthenogenetic one had 24 chromosomes and must therefore be considered triploid. Meiosis in the exceptional triploid ♂♂ showed absence of chromosome pairing. Two ordinary equational divisions could be observed and these led to the development of triploid spermatozoa which did not function at copulation with the triploid parthenogenetic ♀♀. Oogenesis was altered in a similar way. The unreduced egg-cells, however, were able to function and developed without fertilization.

Similar conditions have been described by ARTOM (1931) for *Artemia salina* and by SEILER (1927) for *Solenobia triquetrella*.

Apart from this transition from bisexuality to parthenogenesis in connection with polyploidy it is reported that polyploid animals have the same general characteristics as polyploid plants. The cell-size is increased and often a general gigantism is seen.

*Vertebrate series.* — FANKHAUSER (1938 a) was the first to concern himself directly with this problem, if we disregard the triploid embryos previously met with in connection with other research work (see above). Examining larvae of ♀♀ taken from natural populations, he found triploids and pentaploids in the species *Triturus viridescens* (FANKHAUSER, l. c. and 1940) and triploids and tetraploids in the species *Eurycea bislineata* (FANKHAUSER, 1939 a). The frequency of triploids in the former species was about 1 % and in the latter as high as 10 %. The chromosome numbers could be determined by a cytological examination of an amputated piece of the caudal fin, which had the advantage that the larvae could remain alive after the determination.

FANKHAUSER (1938 a) followed the development of four triploid *Triturus viridescens* larvae up to metamorphosis. The course of development showed nothing remarkable. Only in one case was there a sign of increased body size; the rest were somewhat above average size but within the range of variation of the diploids. The same seems to be the case with the triploid *Eurycea* larvae (FANKHAUSER, 1939 a). No marked difference in general viability was found between the diploid and triploid larvae, though perhaps the latter seem to be more vigorous. On the other hand, the tetraploid larvae seem to deviate from the normal. They are somewhat smaller than the diploid, grow more slowly and show reduced viability. The development of a pentaploid larva (FANKHAUSER, 1940) was at first normal, but after 4 weeks a general oedema began to appear. The larva showed no sign of gigantism. The viability was much reduced.

Thus these investigations already show one important deviation from the majority of polyploid plants, namely the lack of general gigantism. The fact that triploid, tetraploid and pentaploid Urodela do not vary essentially as regards body size from the diploids allows the assumption of some regulatory mechanism. With polyploidy there arises the expected increase in cell-size but not a correlated increase in the size of the organs. Instead, the number of cells per organ decreases so that the organs are of about the same size as those of the diploids (FANKHAUSER, 1939 b).

Proof that triploid Urodela can undergo normal metamorphosis and develop further to adult animals was given later for the species *Triton taeniatus* (BÖÖK, 1940). An adult ♂ of this species was found in a natural population; this one, too, did not deviate essentially from the diploids as regards body size.

All these investigations of vertebrates cannot be covered by a conception expressed by HEILBORN (1934) and DARLINGTON (1937) that an increase of organ size is an obstacle to polyploidy in animals. However, although gigantism is usually the consequence in plants, there are several exceptions and even cases where high chromosomal plants are smaller than low chromosomal (cf. DARLINGTON, 1937, p. 223). Here DARLINGTON says: »It seems as though doubling will increase the size of the normal type in most cases, but that some organisms are more adapted to a specific size so that particular genetic conditions influencing size, such as chromosome number, may have a deleterious effect. This condition is perhaps in part responsible for the general absence of tetraploidy amongst animals».

As regards the Urodela, it seems that the addition of one genome is not accompanied by any considerable disturbance of development or changes in body size. With triploidy, however, it seems that an optimum of polyploidy has already been reached. Tetra- and pentaploidy involve disturbances and a reduction of viability that is not compatible with normal development. Besides, body size also decreases in relation to the diploids when the chromosome number exceeds 3x.

The most important obstacle to polyploidy should be sought in the slight ability of the different organs to vary in size or, expressed otherwise, in their efforts to maintain their original size. Each organ is dependent for its activity on a certain nuclear surface. The diploids must be regarded as the most highly adapted. An increase of the chromosome number is accompanied by an increase of nuclear size. If the organs cannot be increased above a certain maximum, the consequence is a decrease of the total nuclear surface. This in turn involves a disturbance of physiological activity. In the first place sensitive organs may be affected such as the endocrine glands, the central nervous system, the heart muscle, etc. The addition of one new genome and an increase in the nuclear size by about 50 % clearly involve no obvious changes of this kind, but seem to form the limit. In tetra- and pentaploidy this limit seems to have been exceeded and the normal physiology upset. It is highly probable that the most common anomaly, namely the general oedema of the larvae both in pentaploidy and haploidy, is due to a decrease in the total nuclear surface and the pathophysiological conditions connected with it.

*Sex.* — Very little is known as yet of sex determination in the class Amphibia. FANKHAUSER (1938 b) reports that of four triploid *Triturus* larvae one had typical testes, the other three had gonads which resembled ovaries although they were smaller than in normal females. It is thus possible that the development of the testes is influenced by triploidy less than the development of the ovaries. The *Triton* case (BÖÖK, 1940) also supports this view. The testes were completely normal without any sign of intersexuality.

## EXPERIMENTAL POLYPLOIDY.

By inducing polyploidy experimentally a valuable complement would be obtained to the spontaneous polyploids. On several occasions the present writer has made experiments with the colchicine method commonly used in plant genetics. Fertilized egg-cells have been treated

with colchicine solutions of different concentrations. These experiments have so far not given any positive results, which may be due to the egg-membranes being impermeable to the colchicine molecules. This difficulty might be got over by removing these membranes before treatment and keeping the eggs in an isotonic solution of sodium chloride or RINGER's fluid. Such experiments, however, have not yet been made.

Another method used in plant genetics is temperature shocks. Treatment by both low and high temperatures has in several cases resulted in a doubling of the chromosome number. If the shock is given during meiosis, diploid gametes may be formed. In a paper by ROSTAND (1934) it is reported that a similar effect is obtained by cold treatment of eggs from *Bufo vulgaris*. Directly after insemination with sperm from another species (*Rana temporaria*) the eggs were treated with a temperature of 0° C. for 15—20 hours. Several of them then developed normally, and ROSTAND considered these were diploids. Thus the cold shock is assumed to have induced a gynogenesis in these cases, as there was no reason to assume that foreign sperm could fertilize the egg-cells but only »activate» them.

Starting from these experiments FANKHAUSER and GRIFFITHS (1939) carried out a similar series, but with normally inseminated egg-cells of the salamander *Triturus viridescens* common in the U. S. A. Directly after the insemination the eggs were treated at a temperature of + 0,5°—3° C. for 16—26 hours. The aim was to produce polyploids and the result was convincing from the first. Of 35 surviving egg-cells no fewer than 24 developed into triploid larvae. The rest were made up of 10 haploids and only one diploid.

Thus it proved possible for the first time to produce polyploid animals also in the vertebrate series in a relatively simple way. Haploid animals have on several occcasions been previously produced in different ways, though not by cold shocks.

The fact that under similar experimental conditions both haploid and triploid animals have resulted has hampered the interpretation of these experiments. FANKHAUSER and GRIFFITHS (1939) suggested that a temperature factor is responsible for the production of haploid or triploid embryos in such a way that minor differences in the low temperature could have an effect in one or other direction. It seems obvious that the low temperature must in some way interfere with the kinetic course during conjugation and the divisions of nuclei and cells involved in that process. It may also be assumed *a priori* that the effect must

be restrictive or paralysing. A more detailed discussion of this mechanism will follow in a later chapter.

## EXPERIMENTAL TECHNIQUE.

*Triton taeniatus* ($2x = 24$) has been the animal used in all experiments except E4 (*T. cristatus*, $2x = 24$).

The cold treatment took place in a cooled water bath. The temperature was controlled by means of an ordinary contact thermometer. The temperatures given show the adjustment of the contact thermometer. The variations amounted to $\pm 0{,}2°$ C.

The larvae and the organs of the adult animals were in all cases fixed in BOUIN-ALLEN's fluid. As a rule this fixative gave good cytological pictures. The staining was done by the usual gentian-violet method.

For the preliminary determination of the chromosome numbers of the larvae a method described by FANKHAUSER (1938 a) has been used, viz. the examination of the mitoses in the caudal fin.

For cytological studies of the embryos section preparations proved rather unsuitable. The occurrence of masses of yolk grains makes it difficult to obtain a good specimen. Instead, a smear method has been used which is a modification of the aceto-carmine method. This was worked out by SVÄRDSON (not published) and its main points are briefly as follows. Fixation in 50 % acetic acid, preliminary staining in iron aceto-carmine, smearing, microscopical control and final staining, and mounting in Canada balsam. The tissues swell in the acetic acid and can then easily be squeezed out under the cover slip. When the embryos are handled in the same way, the embryonic tissues chiefly move to the periphery, while the yolk stays in the centre. This method, which also has the advantage of being rapid, ought to be very useful for chromosome studies in general. It has also proved to give very good meiotic preparations.

## CASE RECORDS.

Below, a short report is given of the different experiments. A detailed report and the microscopical preparations are available at the Institute of Genetics at Lund.

Experiments marked with the letter Ä are those made with egg-cells, experiments marked with E those with embryos. Each series has its special number, and the index indicates a special individual. For instance, $Ä\,2_6$ indicates individual No. 6 in egg-cell series No. 2. Table 1 is a summary of experiments Ä 2 to Ä 14 b inclusive. In all these series the temperature treatment took place in close connection with the deposition and in general not more than 30 minutes elapsed after insemination. The egg-cells are inseminated at the moment of deposition (cf. BÖÖK, 1941).

Some of these experiments demand more detailed comments than can be elicited from the tables.

Ä 2. The larva $Ä\,2_4$ deviated early from the rest because of its low viability. It then proved to be haploid. A description is found in BÖÖK (1941).

Ä 9 b. Irregular gastrula with cells very different in size. Rapid degeneration in connection with gastrulation.

TABLE 1. *Summary of egg-cells treated with low temperature in close connection with insemination. Embryos within parentheses.*

| Exp. No. | Eggs total | Duration, hours | Temp. °C. | Surviv. larvae | Total age at fixat. in days | Intraovul. developm. in days | Larvae x | Larvae 2x | Larvae 3x |
|---|---|---|---|---|---|---|---|---|---|
| Ä2 | 11 | 7 | 1,2—2,1 | 6 | 13 | 9,3 | 1 | 5 | |
| Ä3 | 3 | 5 | 1,2—1,4 | 1 | 13 | — | | 1 | |
| Ä4 | 3 | 4 | 1,2—1,4 | 2 | 13 | — | | 2 | |
| Ä9a | 7 | 15 | 2 | 7 | 23 | 16,0 | | 7 | |
| Ä9b | 1 | 6 | 2 | — | — | — | | — | |
| Ä10b | 19 | 10 | 2 | — | — | — | | — | |
| Ä11a | 25 | 6 | 2 | 12 | 22 | 14,2 | | 11 | 1 |
| Ä11b | 10 | 8 | 2 | 5 | 22 | 15,6 | (1) | 3 | 2 |
| Ä12 | 27 | 6 | 2 | 7 | 20 | 16,0 | | 5 | 2 |
| Ä13a | 7 | 5 | 2 | — | — | — | (1) | | |
| Ä13b | 2 | 8 | 1 | 2 | 18 | 16,0 | | 2 | |
| Ä14a | 6 | 7 | 1 | 4 | 19 | 15,0 | (1) | 4 | |
| Ä14b | 8 | 9 | 1 | 2 | 18 | 14,0 | | 2 | |
| — | Σ 129 | — | — | Σ 48 | — | — | Σ 4 | 42 | 5 |

Ä 10 b. In most cases irregular cleavage. No embryos survived gastrulation.

Ä 11 a. Egg-laying was induced by hypophysis transplantation. Ten embryos degenerated in connection with gastrulation. Seven showed later an extremely irregular development, in some cases a complete embryonic disorganization and in consequence a rapid degeneration. In one case the embryo divided into two parts, one of which consisted of pure yolk and later degenerated. A miniature embryo developed out of the other part and proved to have the normal diploid chromosome number. One of the larvae (Ä 11 $a_{12}$) was a uniform triploid.

Ä 11 b. Two embryos showed irregular development after gastrulation. The one with complete tissue disorganization degenerated after 6 days. The other showed division with formation of a miniature embryo, which in this case was considerably smaller than normal and oedematous. The chromosome number could not be determined, but probably haploidy was present. One of the other embryos, which for the most part developed normally (diploid), showed a total aplasia of the left eye. Such abnormalities of the eyes are well known from earlier experiments with abnormal treatment during embryogenesis (cf. STOCKARD, 1931). In these experiments they occurred both in haploids and diploids and should thus have no connection with the chromosome number but should be considered as phenocopics. Ä 11 $b_2$ and Ä 11 $b_5$ were normally developed uniform triploids.

Ä 12. Fifteen embryos degenerated before or during gastrulation. In 9 cases normal medullary plates developed. In 3 cases the tissue differentiation was disorganized and these degenerated after 6 days. After 9 days two others degenerated without macroscopically visible irregularities. In two cases (surviving) the development of the eyes was delayed but anlagen were found bilaterally. The cytological

examination showed diploidy in 5 cases (including the two larvae with ocular hypoplasia) and in 2 cases (Ä $12_1$ and Ä $12_3$) uniform triploidy.

Ä 13 a. Hypophysis transplantation. One egg-cell developed into a short, thick and oedematous embryo, which degenerated after 10 days. Probably haploidy was present.

Ä 14 a. One embryo degenerated in connection with gastrulation. Another showed a somewhat irregular medullary plate. The body of this embryo then became short, thick and oedematous. A large blister filled with fluid developed near the heart. It was fixed after 11 days *intra vitam*. Microscopical examination showed haploidy.

Ä 14 b. Five embryos degenerated at gastrulation. One showed an irregular medullary plate and degenerated at the stage of neural fold.

*Controls.* — 71 egg-cells, all laid spontaneously. 8 of these degenerated before or in connection with gastrulation. 63 developed into normal larvae, which were all diploid. The duration of the intra-ovular development varied between 8 and 16 days.

It could thus be established as a preliminary fact that a cold shock applied to an unsegmented egg-cell very soon after insemination (in most cases within 30 minutes) could in certain cases induce both haploidy and triploidy as well as several aberrations in the embryogenesis. Further, such a cold treatment is also consistent with a normal diploid development. It was also interesting to be able to confirm that such a short treatment as was given here and applied to the still undifferentiated egg-cell could induce an abnormal development. Eye anomalies have been put forward as examples. These are not connected with changes in the chromosome number and probably their cause has no connection at all with chromosomal conditions.

As regards the intraovular course of events giving rise to the haploid and triploid embryos, no definite conclusions can be drawn from the results of these experiments. In the investigations made by GRIFFITHS (1941) it was shown that haploidy or triploidy could not be induced if the temperature treatment was begun later than 30 minutes after the deposition (= insemination). The question was therefore in the first place: Is this time factor also relevant for *Triton* and, if so, in what way?

To shed some light on this question a series of experiments was made in which the cold shock was applied when certainly more than 30 minutes had elapsed after insemination but before the first cleavage. Table 2 gives a summary of these experiments.

Ä 16. Two embryos developed normally. Fixation after 3 days. Cytological examination of embryo Ä $16_2$ showed cells with 26, 28 and $29 \pm 2$ chromosomes. No metaphases with the normal diploid number could be found.

TABLE 2. *Diagram of egg-cells treated with low temperature (+ 2° C.) later than $^1/_2$ hour after insemination.*

| Exp. No. | Total | Duration, hours | No cleavage | Abnormal cleavage | Surviving after 3 days |
|---|---|---|---|---|---|
| Ä 16 | 7 | 3 | 5 | — | 2 |
| Ä 17 | 3 | 5 | 3 | — | — |
| Ä 18 | 5 | 6 | 4 | — | 1 |
| Ä 19 | 2 | 7 | 1 | 1 | — |
| Ä 20 | 16 | 7,5 | 8 | — | 8 |
| Ä 21 | 8 | 16 | 8 | — | — |
| Ä 22 | 8 | 8 | 6 | 2 | 2 |
| Ä 23 | 15 | 14 | 15 | — | — |
| | 64 | | 50 | 3 | 13 |

Ä 19. One egg-cell divided into a number of cells extremely different in size and then degenerated.

Ä 20. Eight egg-cells showed normal cleavage and development. Five embryos were examined cytologically after 3 days. The chromosome numbers found are shown in Table 3. The embryo Ä $20_3$ showed the greatest irregularities. Coincident

TABLE 3. *Chromosome numbers found in multiform aneuploid embryos.*

| Embryo No. | Chromosome numbers |
|---|---|
| Ä $16_2$ | 26, 28, 29 ± 2 |
| Ä $20_1$ | 17, 24 |
| Ä $20_2$ | 16, 24, 27 |
| Ä $20_3$ | 11, 12, 13, 14, 18, 26, 27, 31, 33 |
| Ä $20_5$ | 28 ± 2, 30 ± 2 |
| Ä $20_6$ | 30 ± 2, 33 |

with the differences in chromosome number of different nuclei an extremely abnormal variation in size could be noted between the nuclei. There were also other irregularities. Fig. 1 shows a specially clear case of a chromosome bridge with a fragment in a mitotic anaphase. Here it is seen that both the fragment and the two lagging chromosomes show a lengthwise split. Whether this is an artefact or a sign that the chromosomes have already »divided» cannot be definitely settled. The phenomenon has been earlier shown in *Amblystoma* (DEARING, 1934) and *Crocus sativus* (GATES and PUTHAK, 1938). DARLINGTON (1938) considers that it is an artefact.

Ä 22. Two egg-cells divided into cells of extremely different size and degenerated.

The present writer proposed the term multiple aneuploidy (BÖÖK, 1943) for these embryos with a varying number of chromosomes in each individual; the term *multiform aneuploidy* is perhaps better.

*Controls of experiments Ä 16 to Ä 23 inclusive.* — Eight 3-days-old embryos were examined cytologically by the same method. They were all normal diploids.

**Fig. 1. Mitotic bridge and fragment. — Figs. 2 and 3. *T. taeniatus* and *cristatus* respectively. Breakdown of centromere orientation after 8 hours at + 2° C. Note the strong chromosome contraction in *cristatus*. — Fig. 4. *T. cristatus*. Centromere division at metaphase. $x_1x_1$ etc. corresponding chromatids. — Fig. 5. *T. taeniatus*. 14 hours after treatment. »Anaphase-telophase« in cold mitosis.**

Altogether 8 endomitotic metaphases with 24 + 24 chromosomes were found. These plates showed definite somatic pairing. If this endomitotic phenomenon is more closely studied, it is found that *the centromere division can be traced to very early prophase stages.* Possibly it already takes place during the interphase. It must be mentioned that in these endomitotic metaphases *all* the chromosomes are centromere-divided and the daughter chromatids lie in pairs.

*Thus a cold shock applied when more than 30 minutes have elapsed after insemination gives in no case uniform haploidy, diploidy or triploidy.* Considering the course of normal fertilization and the first cleavage (see further below) it has been shown to be probable that the internal constitution of the egg-cell is of essential importance for the final result of the cold shock.

It is thus clear that to induce *balanced* changes in the chromosome number the cold treatment should be applied in close connection with the fertilization process. By artificial fertilization complete control could be obtained over the point of time at which it occurs. Yet the point of time for the conjugation of the gamete nuclei cannot be exactly determined. However, there seems to be great difficulties in carrying out artificial fertilization of *Triton.* So far only one such experiment has given a positive result.

Ä 29. 78 egg-cells were excised from the lower part of the oviduct. 50 of these were inseminated with sperm from ductus deferens (active movement of the spermatozoa). Duration of insemination 30 minutes at the most. Immediately afterwards cold treatment + 2° C. for 8 hours. Of these 50 eggs only one developed (completely normally) and was fixed after 7 days. Cytological examination showed uniform triploidy. Of the other 28 non-inseminated and untreated egg-cells none showed any sign of cleavage.

In this experiment there is every reason to assume that conjugation had not taken place when the cold treatment began. The result was a triploid embryo.

In order to decide exactly what happens within the egg-cell during and after the temperature treatment it would naturally be desirable to make a cytological examination at the same time. This, however, comes up against considerable technical difficulties, owing chiefly to the abundance of lipoids in the egg-cell.

Starting from the assumption that the low temperature must in some way affect the mechanism of cell division, this effect could also be studied at embryonal stages, which are cytologically more easily accessible. It is most likely that the effect would be the same whether the first cell division be studied or a later one. In any case a clue ought

to be obtainable by examining the effect on the later cell divisions. For this reason the following experiments were made.

E 1. Four embryos normally developed and at the neurula stage were treated with a temperature of + 2° C. for 8 hours. Fixation 5 hours later. The cytological examination showed, besides metaphases of normal appearance, some which were a kind of intermediate stage between normal and endomitotic plates. Thus, in several cases, some of the metaphase chromosomes were divided at their centromeres without showing any sign of repulsion (cf. BÖÖK, 1943, Fig. 1). These metaphases deviate essentially from the endomitotic plates already mentioned. Their structure gives reason to assume that the centromere division in these cases takes place at metaphase and that the absence of normal repulsion is a consequence of the cold shock.

E 3. 18 embryos, 5 days old, cold treatment + 2° C., 8 hours. After treatment fixation successively with one hour's interval. Directly after the treatment plenty of metaphases and prophases in different stages were found, but no anaphases. The orientation of the centromeres seems to be disturbed at metaphase (cf. Figs. 2—5). Transitions from complete irregularity via even distribution in the plate (the common plant type) to the normal hollow spindle. After two hours anaphases again appeared. Plates with 48 chromosomes without somatic pairing. After 14 hours restitution nuclei with 48 chromosomes.

E 4. *T. cristatus.* This experiment was arranged in the same way as E 3. The cytological examination showed about the same circumstances as in the previous experiment. In some of the metaphases the chromosomes, immediately after treatment, were abnormally contracted, recalling the meiotic ones (double spiral?). This reaction was not observed in *T. taeniatus.*

## EFFECT OF LOW TEMPERATURE ON CELL DIVISION IN EMBRYOS.

In interpreting experiments E 1 to E 4 we have to consider the following facts:

(1) During the cold treatment the movements of the chromosomes are slowed down. At + 2° C. there is probably almost absence of motion.

(2) The centromeres lose more or less the ability to orientate themselves to a normal metaphase plate.

(3) The centromere division can take place at + 2° C., although at a slower pace.

(4) Later in the subsequent course restitution nuclei are found.

(5) In *T. cristatus* there is an abnormally strong contraction, which is not otherwise seen in mitotic chromosomes.

These facts may now be brought into agreement with an effect upon the spindle apparatus. If the development of this is prevented at + 2° C., the metaphase stage cannot be passed. That explains why

so many metaphases are found immediately after the treatment. Then gradually the situation again becomes normal and anaphases reappear.

A regular orientation of the centromeres implies an unimpaired balance: centrosome—spindle—centromere. This is disturbed if the spindle is unable to function and the orientation fails. Different degrees of disturbed balance are found here. In *Triton* there is normally a so-called hollow spindle, i. e. the chromosomes are distributed round the edge of the plate. According to DARLINGTON (1937, p. 525), this depends on a stronger polar repulsion. Further, he points out: »When this is paralysed by low temperature it will influence the distribution of the chromosomes and the first change will be a congression of the chromosomes to a more or less homogeneous plate. Later on they may be irregularly crowded». It is an open question whether this stronger polar repulsion is due to a characteristic of the spindle or of the centrosomes. In any case the description fits exactly some of the cold-treated metaphases in this material (cf. Figs. 2—5). The stronger contraction of the chromosomes in *cristatus* (cf. Fig. 3) is probably also connected with a paralysis of the spindle (DARLINGTON: »external mechanism», 1937, p. 512). In this way a longer period would be available for spiralization.

In the embryos examined directly after treatment centromere division at metaphase was also found without these centromeres repelling each other as in normal cases (cf. Fig. 4). This can scarcely be interpreted in any other way than that the repelling forces (spindle, external mechanism or whatever they may be called) are unable to function, but that in spite of this the contact between the chromatids gradually ceases. The cell in this way receives a double number of chromosomes. An indication of the formation of restitution nuclei with this number, 48, is certainly due to the fact that when once the spindle is put out of action it cannot be regenerated in the same division phase.

Thus everything indicates that the most important effect of the temperature treatment here discussed consists in a breakdown of the spindle mechanism. On the other hand, the centromere mechanism seems to remain intact. Using DARLINGTON's terminology, we should say that the external mechanism of the chromosomes is more sensitive to temperature changes than the internal one.

## EFFECT OF LOW TEMPERATURE ON THE UNSEGMENTED EGG-CELL.

Organic life is possible only within relatively limited temperatures. Temperature changes in the environment in which the vital processes

take place must therefore have a decisive effect on their course. In a general way it is known that an increase of temperature up to a certain maximum intensifies the physiological processes while a decrease has a restrictive effect. As the molecular movements in a gas or a liquid decrease the speed of the movements in the cell also decreases as the temperature falls. Thus it can be observed that related movements like the cytoplasmic currents may be stopped at temperatures near 0° C. (SINKE, 1939; *Tradescantia virginica*). The most interesting thing in this connection is the effect of subnormal temperatures on the cell-division mechanism. BARBER (1939) has examined the anaphase movements in *Tradescantia* under different temperature conditions. Although in these experiments no extreme temperatures were used, a slowing down of the anaphase movements could be clearly observed at + 10° C. The same thing happens with fibroblasts of *Gallus domesticus*, cultivated at + 21° C. (BUCCIANTE, 1927; cf. BARBER, l. c.). In agreement with this it has been found that the speed at which the egg-cleavage takes place in *Ascaris* decreases when the temperature is lowered (cf. BARBER, l. c.).

Now the question is whether this slowing down of the anaphase movements at subnormal temperatures depends upon an increased cytoplasmic resistance, i. e. an increased viscosity, or upon a concurrent effect of some kinetic organ within the cell. According to BARBER (l. c.), it seems that changes in the viscosity are of no importance, chiefly because the movements of the chromosomes in relation to those of the other cell constituents are so slow that it is highly improbable that a change in viscosity would influence these movements. The temperature therefore seems to affect the cell division in some other way than through viscosity changes.

Before going further it is necessary to mention the course of normal fertilization in *Triton*. As a rule, the egg-cell at insemination is at the second metaphase stage of meiosis. Several spermatozoa penetrate at the same time, i. e. a physiological polyspermy is the rule. Only one spermatozoon conjugates with the egg-nucleus after the formation of the second pole-cell. The others degenerate after a short time. As it is unreasonable to assume that the cold shock involves the participation of another spermatozoon in conjugation, these superfluous spermatozoa can be ignored.

As triploidy, haploidy and multiform aneuploidy can arise under the same temperature conditions, it must be assumed that *the internal constitution of the egg-cell* and not minor temperature differences *is*

*decisive for the result.* Probably a time factor in the egg-cell's changes determines the effect.

Within the first half-hour after insemination the following events

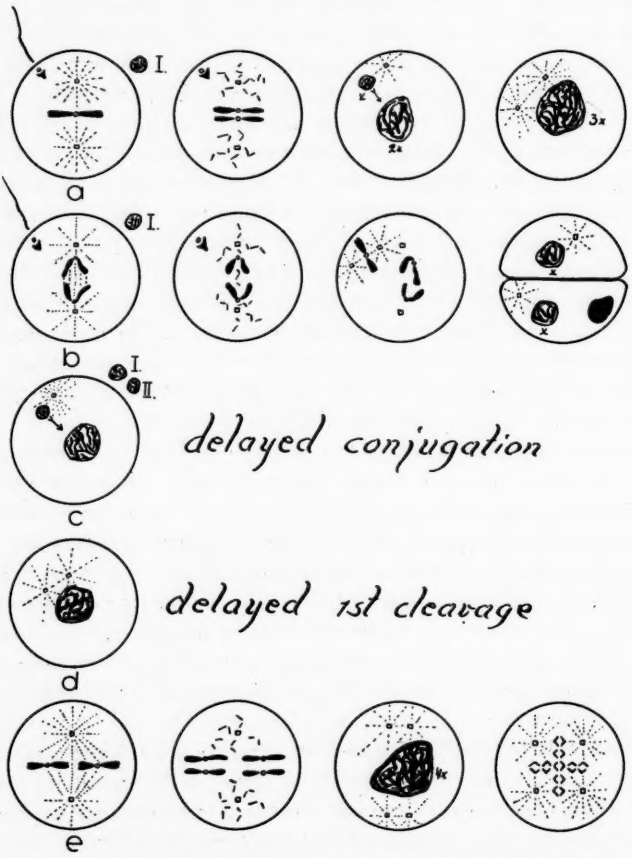



Fig. 6. Diagram of probable events after cold treatment of the fertilized egg-cell at different stages leading to triploidy, haploidy and multiform aneuploidy. Left vertical *a*—*e* the normal stages. Cold treatment of *c* and *d* should yield normal diploid development. Further discussion in text.

may be assumed to take place within the egg-cell (cf. Fig. 6). The sperm-nucleus with its centrosome penetrates the egg while the latter's second meiotic division is taking place. The second pole-cell is formed. The two haploid nuclei conjugate and form the zygote-nucleus. The

centrosomes divide while the zygote-nucleus enters upon its first mitotic prophase. So several conditions of the time factor are given during which a momentary cold shock can have different effects.

A moderate lowering of the temperature involves, as has been stated above, a retardation of all movements in the cell. A rapid reduction to + 2° C., on the other hand, probably involves a complete paralysis of the spindle (cf. also CALLAN, 1942). The consequence of this would be a blocking of the cell division at the metaphase stage.

In plant material EHRENBERG and ÖSTERGREN (1942) found that multipolar spindles are common at 0° C. (*Salix*). Analogous to this is the finding by CALLAN (l. c.) of abnormal spindles in *Triton* larvae at + 3° C. Judging from these investigations it therefore seems that the spindle apparatus is the mechanism which is affected above all by a fall in the temperature. It seems that as the temperature is lowered it is possible to obtain all transition stages from a retardation of the spindle formation via abnormal spindles to complete paralysis.

As the spindle »fibres» may be assumed to arise by means of the formation of molecular chains (DARLINGTON, 1937), there is theoretically no obstacle to the idea that low temperatures render this difficult or disorganize it. In animals the centrosomes are also included in the spindle apparatus, and this fact must be considered because they serve as a kind of organizer for it. Whether the centrosomes also exist in higher plants is not yet clear; in any case their existence cannot be cytologically proved. In animals they seem partly to affect the configuration of the spindle and partly to form some kind of a kinetic organ. Thus more than two centrosomes give rise to multipolar spindles. If, on the other hand, in a normal cell reproduction of the centrosomes is prevented, cell division does not occur and a restitution nucleus is formed. This has been convincingly shown by BOVERI in his well-known experiments with egg-cells of echinoderms (cf. DARLINGTON, 1937). DARLINGTON (l. c.) comes to the conclusion that »the centrosome is a self-propagating body, controlling the formation of the spindle». The reproduction of the centrosome normally takes place during the interphase and in all circumstances before the mitosis has reached the metaphase stage (cf. WILSON, 1928).

Centrosomes, spindle »fibres» and centromeres form centres and fields of force during cell division. It is now of considerable interest that the spindle proper, i. e. the spindle-»fibres», seem to assume a special position, while the centrosomes and the centromeres seem, so to say, to be closely related to each other (cf. DARLINGTON, 1937). Im-

portant support for this idea may be obtained in the experiments made by VAN BENEDEN, RABL, BOVERI *et alii* (cf. WILSON, 1928, p. 175). Both cell division and nuclear division can be prevented by treatment with ethyl ether, while centrosome and centromere reproduction continues. Thus the number of chromosomes and centrosomes increases and giant nuclei arise, surrounded by a large number of centrosomes. When the ether narcosis comes to an end multipolar mitoses appear. The development of the spindle-»fibres» is thus the phase which is primarily attacked by paralysing agencies.

As is already known, the centrosome of the egg-cell is eliminated in connection with fertilization. This elimination takes place after the second meiotic division when the ripe egg-nucleus has been formed. How it takes place is, however, quite obscure. The phenomenon is possibly analogous to the inactivating of superfluous centrosomes in normally polysperm eggs.

On the basis of the facts and deductions presented here a conception now may be formed of the mechanism which gives rise to *uniform triploidy in Triton* under the effect of low temperature. It is assumed that the cold shock strikes the egg-cell immediately after insemination. Its appearance corresponds roughly with Fig. 6 *a*. The spindle is broken down by the sudden application of the low temperature (+ 2° C.), while both the sperm centrosome and the centromeres are affected less destructively. A transition from metaphase to anaphase is in this way prevented and the chromosomes remain lying in metaphase configuration. The spindle is now definitely out of action and cannot be reorganized. The chromosomes still lie for a while in metaphase configuration and now the centromeres divide. This metaphase, probably after treatment, then passes over without anaphase into a restitution nucleus with a doubled number of chromosomes. Thus, in this case it becomes diploid.

The egg-cell now contains a diploid egg-nucleus with two centrosomes and the haploid sperm-nucleus with its centrosome. Conjugation takes place normally and a triploid zygote-nucleus is formed. The centrosomes of the egg-nucleus are put out of action in the usual way and the embryo is developed out of this balanced autotriploid zygote.

The course of events leading to the *rise of haploids* after temperature treatment may be reviewed according to the same principle if only it is assumed that the time factor of the egg-cell is a different one. It must be assumed *a priori* that for some reason there has been no conjugation between the two gamete-nuclei. This may be due to

the fact that the cold shock struck the egg-cell at a somewhat later stage, when the second meiotic division was in anaphase (Fig. 6 *b*). The spindle is put out of action and the division is blocked with the chromosomes lying in anaphase configuration. As the temperature rises to the normal after treatment, the anaphase movement cannot be started again, in any case not immediately. It must be assumed that this anaphase grouping has no attraction for the sperm-nucleus, which therefore remains where it was when the treatment started. A block in anaphase also prevents a sufficiently rapid formation of a restitution nucleus or of two separate nuclei. Being less affected by the low temperature than the spindle, the sperm centrosome within a relatively short time after the treatment can divide and form a spindle around the haploid sperm-nucleus. The latter thus manages to achieve a division configuration before the disorganized second anaphase has produced any fertilizable nucleus. Thus the embryo starts its development with the haploid paternal set of chromosomes.

Besides this probable course of events, which should give rise to triploid and haploid embryos capable of further development, it is natural that other more irregular events may also occur as an effect of the cold shock applied within half an hour of insemination. Thus, according to the first alternative (the rise of 3x embryos), embryos with unbalanced chromosome numbers may also be thought to arise. One or several undivided chromosomes may be included in the restitution nucleus and not divide until the next mitosis. Another possibility is that individual chromosomes may be disorientated and eliminated when the combining force is lost through the destruction of the spindle. This may explain the high mortality and some of the irregularities in the embryogenesis which have been observed here.

A quite deviating result is obtained if the cold shock is applied when more than half an hour has elapsed after insemination. The mortality increases still further (cf. Table 2) and those embryos which have actually begun their development degenerate sooner or later. Such embryos show interesting chromosome conditions. One and the same embryo contains cells with different chromosome numbers (see above). These different numbers can be explained in no other way than by the occurrence of multipolar divisions at an early stage.

The production mechanism of *multiform aneuploid embryos* may be explained in the following way. If the cold shock strikes the egg-cell after the conjugation has taken place normally, it may happen that the first mitotic division is blocked at the metaphase stage (cf. Fig. 6 *e*).

The effect is analogous to the previous one in that a tetraploid restitution nucleus is formed. Meanwhile the centrosomes also divide and the consequence is that multipolar divisions arise later. This omission of a nuclear and cell division while retaining the division rhythm of the centrosomes and the chromosomes might explain the rise of these multiform aneuploid embryos.

Another interesting feature in these experiments deserves mention. Of the 64 egg-cells treated, no fewer than 50 degenerated without showing any sign of cleavage. The cold shock can thus directly destroy the division mechanism in such a way that further development is rendered impossible. This fact may further support the production mechanism for haploids outlined above.

It is worth noticing that such embryos, in spite of their unbalanced cytological circumstances, can in some cases gastrulate normally. They have been followed up to the neurula stage, and some of them, macroscopically examined, showed no deviation from the normal. Evidently the early differentiating mechanism is not absolutely bound to balanced chromosome numbers in *Triton*. Yet sooner or later these embryos degenerate.

Quite recently some of the results communicated here have been confirmed in an excellent way in another material. Thus SVÄRDSON (personal communication), examining Salmonid embryos which had been exposed to low temperature, found both haploids, triploids and embryos with multiformly unbalanced chromosome numbers. Probably a similar mechanism underlies the origin of these embryos.

One fact which has not yet been touched upon is that in spite of the cold shock normal diploids may develop. The explanation seems simple. If the egg-cell is at the stage shown schematically in Fig. 6 *c* or *d*, the low temperature cannot affect any spindle but only the two gamete-nuclei or the zygote-nucleus and delay conjugation or first cleavage. As a matter of fact, the hypothesis worked out here demands the presence of normal diploids in the treated material.

We have thus reached the conclusion that, besides the temperature and its duration, *the internal constitution of the unsegmented egg-cell at the start of the experiment is of decisive importance for the result of the cold shock*. The rise of haploidy, triploidy and multiform aneuploidy can in a simple way be ascribed to a destructive effect on the spindle, while the centrosomes and the centromeres are affected to a less degree.

## C-MITOSIS AND COLD MITOSIS.

The effect of low temperature on the cell-division mechanism leads to a comparison with a similar effect obtainable with other agencies which also and mainly affect the spindle apparatus. Those that have been best examined are colchicine and acenaphthene. The term c-mitosis was introduced by LEVAN (1938) for the type of mitosis induced by treatment with colchicine.

Shortly described, the effect of colchicine consists in an inactivation of the spindle apparatus and a retardation of the centromere division (LEVAN, l. c.). As an expression of this latter effect characteristic so-called c-pairs appear at metaphase. The chromosomes are attached only at their undivided centromeres. These c-pairs usually appear a few hours after the start of the colchicine treatment. Later, the centromeres divide and the daughter chromatids are found lying in pairs. It is interesting that in the so-called c-anaphase some chromosomes can be found with undivided and others with divided centromeres. This is undeniably reminiscent of conditions in some of the metaphases described in experiments E 1—E 4 (see above). Obviously in both cases there is a desynchronizing of the centromere division.

The c-mitosis and the cold mitosis thus resemble each other, both being the result of a breakdown of the spindle mechanism. The way in which the disorganization is brought about may, however, be different, as in the one case a purely chemical factor is introduced, in the other a physical one.

In the colchicine experiment the centromere division can take place in spite of the fact that the cell is under the influence of the colchicine. The chromosome number may be successively increased from 2x to 4x and so on without any cell division taking place. Not until the effect of the colchicine comes to an end do cell divisions with *normal* spindles again occur. This latter circumstance may indicate that centrosomes are lacking in higher plants (*Allium* is the material referred to here). If the centrosomes could also reproduce themselves under colchicine treatment, one must *a priori* expect multipolar spindles after the treatment. If, on the other hand, they do exist, here is an essential difference between them and the centromeres.

As far as can be judged from the literature (cf. RIES, 1939; CHODKOWSKI, 1937), the effect of colchicine on *animal cells* has not been the subject of any exact cytological investigation. Instead, research-workers have become involved in the question whether colchicine

stimulates mitosis or not. This question, however, is of secondary interest. The essential thing is to examine how the colchicine interferes with the mitotic mechanism. It seems that colchicine does not affect animal mitoses in the same plain way as those of the plants, which may explain the different prevalent opinions. A prominent characteristic is that the effect consists mainly of *irregularities and pyknoses.* Presumably *isolated tetraploid cells* have also been observed, but as far as the author is aware no tetraploid tissue has been discovered. KEPPEL and DAWSON (1939) report that the mitoses were very irregular after the colchicine treatment and that there were considerable variations in the size of the cell-nuclei (*Rana pipiens* embryos).

These results could be looked upon from the same angle as the cold experiments described above which gave rise to multiformly aneuploid embryos. The discrepancy between the colchicine effect in animals and plants may be due to differences in the constitution or activity of the centrosomes *or* to their absence in the higher plants. If, as seems probable in the animal cell, too, the spindle is put out of action by the colchicine while the centromeres *and the centrosomes* are able to divide before a new spindle can be formed after the treatment, then the consequence must be disturbances in the mitotic mechanism. As a result of this pyknoses may also occur. *Hence the induction of polyploidy in animals by means of colchicine treatment becomes very dubious.*

The metaphase blocking is obviously complete in both cases so long as the treatment lasts. Yet with a weaker concentration of colchicine a less definite effect on the spindle is obtained with varying irregularities in consequence. With other temperature conditions similar results can be obtained, which are presumably transition forms to the total block (see above).

The most interesting similarity between c-mitosis and cold mitosis lies in the fact that the mechanism in the spindle which produces anaphase movements is affected in a similar way. Evidently it is this which is most sensitive to noxious influences and most easily put out of action.

In parenthesis a parallel may be drawn to human pathology, namely cancer, which is partly a cytogenetical problem. It seems possible to find a simple explanation for the appearance of abnormalities in the spindle mechanism and the polyploid cells in the tumour tissue (cf. ANDRES, 1932). In connection with the complex changes taking place here with an accumulation of abnormal products of metabolism, etc., it is not to be wondered at that the susceptible spindle may be

damaged. Further support is found for the assumption that these abnormalities are of a secondary nature.

A comparison between cold effect and acenaphthene effect seems superfluous, as the difference between the influence of this substance and colchicine lies only in the different speeds of the c-mitosis. LEVAN (1940 b, p. 273) says: »The acenaphthene effect, as it appears in *Allium*, may consequently be regarded as an ultra-rapid exposure of colchicine action».

## THE EXPERIMENTAL TRIPLOID LARVAE.

The addition of one new genome in *Triton* does not involve any macroscopical change in the embryogenesis. On account of the latter's course it is not possible to differentiate between diploid and triploid animals. For a clear distinction it is necessary to determine the chromosome numbers and then only it may be possible to say that the triploids are somewhat more vigorous than the diploids. In no case was there an obvious increase of body size. There is thus full agreement between this and the circumstances in *Triturus viridescens* and *Eurycea bislineata* (cf. the literature quoted above).

The five triploid larvae were fixed *intra vitam* when 20—22 days old and examined cytologically. They all proved to be uniform triploids. The chromosome number was 36 in all the tissues examined.

*Triploid nuclear volume.* — The size of the cell-nuclei in triploid Urodela has not previously been measured. FANKHAUSER (1939 b) only reports that they are considerably larger than in the diploids.

It is not possible to determine exactly the nuclear volume in so far as it is not a question of spherical nuclei (e. g. spermiocytes and prespermatids). One must be satisfied with a compromise, but this is irrelevant, as here we have to do with relative quantities, i. e. a comparison between the nuclear size in diploids and triploids.

The cell-nuclei have been drawn by means of a camera lucida (ocular 12 and objective 90). No selection was made. The largest ($2\,a$) and the smallest diameters ($b$) were measured in millimetres and the products $ab^2$ were used as comparative quantities. Thus the calculation is based upon the formula of the ellipsoid of revolution. This method has been used to try to compensate in some degree for a possible change in the nuclear form when the new genome is added. The haploids have a more spherical nuclear form than the diploids (BÖÖK, 1940). However, it appeared later that no definite difference could be proved in nuclear form between diploids and triploids.

TABLE 4. *Nuclear size of triploid erythrocytes.*

| Larvae No. | $M_{ab}^2$ 100 | 200 | 300 | 400 | 500 | 600 | 700 | n | M ± m |
|---|---|---|---|---|---|---|---|---|---|
| Ä 11 $b_2$ | 15 | 41 | 41 | 3 | | | | 100 | 282 ± 8 |
| Ä $12_1$ | 1 | 24 | 35 | 24 | 13 | 3 | | 100 | 383 ± 11 |
| Ä $12_3$ | | 25 | 36 | 23 | 14 | 2 | | 100 | 382 ± 11 |
| | 16 | 90 | 112 | 50 | 27 | 5 | | 300 | (349) |

$P < 0{,}001$

*Nuclear size of diploid erythrocytes.*

| Larvae No. | $M_{ab}^2$ 100 | 200 | 300 | 400 | 500 | n | M ± m |
|---|---|---|---|---|---|---|---|
| Ä 9 $a_5$ | 52 | 45 | 3 | | | 100 | 201 ± 6 |
| Ä 9 $a_7$ | 74 | 24 | 2 | | | 100 | 178 ± 5 |
| Ä 11 $a_2$ | 38 | 60 | 2 | | | 100 | 214 ± 5 |
| Ä 11 $a_8$ | 67 | 30 | 3 | | | 100 | 186 ± 5 |
| Ä $12_6$ | 26 | 41 | 31 | 2 | | 100 | 259 ± 8 |
| Ä $15_2$ | 46 | 48 | 6 | | | 100 | 210 ± 6 |
| | 303 | 248 | 47 | 2 | | 600 | (208) |

$P < 0{,}001$

*Comparison of nuclear volume in diploid and triploid.*

Erythrocytes.

| | $M_{ab}^2$ 100 | 200 | 300 | 400 | 500 | 600 | 700 | n | M |
|---|---|---|---|---|---|---|---|---|---|
| 6 diploids | 303 | 248 | 47 | 2 | | | | 600 | (208) |
| 3 triploids | 16 | 90 | 112 | 50 | 27 | 5 | | 300 | (349) |

Average nuclear volume ratio 2n : 3n = 1 : 1,68

TABLE 5. *Nuclear size of cells from cerebral ganglia in diploids.*

| Larvae No. | $M_{ab}^2$ 300 | 500 | 700 | 900 | 1100 | 1300 | n | M ± m |
|---|---|---|---|---|---|---|---|---|
| Ä 9 $a_5$ | 3 | 63 | 29 | 5 | | | 100 | 672 ± 12 |
| Ä 9 $a_7$ | 5 | 70 | 23 | 2 | | | 100 | 644 ± 12 |
| Ä 11 $a_2$ | 4 | 30 | 52 | 10 | 4 | | 100 | 760 ± 16 |
| Ä 11 $a_8$ | 7 | 61 | 31 | 1 | | | 100 | 652 ± 12 |
| Ä $12_6$ | 5 | 36 | 48 | 11 | | | 100 | 730 ± 14 |
| Ä $12_7$ | 1 | 39 | 49 | 8 | 3 | | 100 | 746 ± 14 |
| Ä $15_2$ | 9 | 59 | 29 | 3 | | | 100 | 652 ± 14 |
| | 34 | 358 | 261 | 40 | 7 | | 700 | (691) |

$P < 0{,}001$

*Nuclear size of cells from cerebral ganglia in triploids.*

| | $M_{ab}^2$ | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Larvae No. | 500 | 700 | 900 | 1100 | 1300 | 1500 | 1700 | 1900 | n | M ± m |
| Ä 11 $b_2$ | | 13 | 35 | 36 | 10 | 5 | 1 | | 100 | 1124 ± 22 |
| Ä $12_1$ | 4 | 24 | 36 | 19 | 14 | 3 | | | 100 | 1048 ± 24 |
| Ä $12_3$ | 1 | 6 | 30 | 29 | 21 | 10 | 3 | | 100 | 1210 ± 24 |
| | 5 | 43 | 101 | 84 | 45 | 18 | 4 | | 300 | (1127) |

$P < 0{,}001$

*Comparison of nuclear volume in diploids and triploids.*

Nuclei of cerebral ganglia.

| | $M_{ab}^2$ | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 300 | 500 | 700 | 900 | 1100 | 1300 | 1500 | 1700 | 1900 | n | M |
| 7 diploids | 34 | 358 | 261 | 40 | 7 | | | | | 700 | (691) |
| 3 triploids | | 5 | 43 | 101 | 84 | 45 | 18 | 4 | | 300 | (1127) |

Average nuclear volume ratio $2n : 3n = 1 : 1{,}63$

The results of these calculations are summarized in Tables 4—7. The statistical calculations have been made according to the method of the analysis of variance (cf. BONNIER and TEDIN, 1940).

It is at once seen that no nuclear size can be demonstrated which is characteristic of diploid nuclei on the one hand and of triploid on the other. The relation between chromosome number and nuclear size is complicated. The nuclear size is determined not only by the chromosome number but also by a series of other factors such as special genes and environment (cf. DARLINGTON, 1937, p. 20). Thus considerable individual variability in size is found both in the diploid and triploid series. Consequently, one is not immediately entitled to combine the average values of the different individuals and thus form an average value for diploid *nuclei* and another for triploid and compare them. However, it may be considered justifiable to refer the different average values direct to the individuals. Thus, as regards the erythrocytes, it is found that the diploid *individuals* have an average nuclear size of 208 units, while the corresponding figure for the triploids is 349 units. In this way the comparison of size shows that the triploid larvae have on the average 68 % larger erythrocytes than the diploid. According to the same reasoning, the cartilage-nuclei of the triploids are 74 % larger and the nerve-cell nuclei 63 % larger than the corresponding nuclei of the diploids (see tables). The variation in size is greatest for the cartilage-nuclei and smallest for the erythrocytes-nuclei (intraclass and interclass variance).

TABLE 6. *Nuclear size of triploid cartilage-cells.*

| Larvae No. | 500 | 750 | 1000 | 1250 | 1500 | $M_{ab}^2$ 1750 | 2000 | 2250 | 2500 | 2750 | 3000 | n | M ± m |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ä 11 $a_{12}$ | | | 6 | 26 | 18 | 27 | 13 | 5 | 4 | | 1 | 100 | 1405,0 ± 40,0 |
| Ä 11 $b_2$ | | 1 | 15 | 19 | 25 | 20 | 12 | 5 | 2 | 1 | | 100 | 1400,0 ± 40,0 |
| Ä 11 $b_5$ | | 1 | 4 | 5 | 17 | 7 | 7 | 3 | 2 | | 1 | 47 | 1520,0 ± 65,0 |
| Ä $12_1$ | | 6 | 34 | 26 | 15 | 8 | 5 | 4 | 2 | | | 100 | 1190,0 ± 40,0 |
| Ä $12_3$ | | | 9 | 13 | 36 | 28 | 11 | 2 | 1 | | | 100 | 1302,5 ± 30,0 |
| | | 8 | 68 | 89 | 111 | 90 | 48 | 19 | 11 | 1 | 2 | 447 | (1405,7) |

$P < 0{,}001$

*Comparison of nuclear volume in diploids and triploids.*

Nuclei of cartilage cells.

| | 250 | 500 | 750 | 1000 | 1250 | 1500 | 1750 | 2000 | 2250 | 2500 | 2750 | 3000 | n | M |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 7 diploids | 3 | 217 | 323 | 124 | 26 | 5 | 1 | | | | | | 700 | (805) |
| 5 triploids | | 8 | 68 | 89 | 111 | 90 | 48 | 19 | 11 | 1 | 2 | | 447 | (1405,7) |

Average nuclear volume ratio 2n : 3n = 1 : 1,74

TABLE 7. *Nuclear size of diploid cartilage cells.*

| Larvae No. | $M_{ab}^2$ 250 | 500 | 750 | 1000 | 1250 | 1500 | 1750 | 2000 | n | M ± m |
|---|---|---|---|---|---|---|---|---|---|---|
| Ä 9 $a_5$ | 1 | 51 | 38 | 9 | 1 | | | | 100 | 765 ± 17,5 |
| Ä 9 $a_7$ | | 41 | 45 | 14 | | | | | 100 | 807,5 ± 17,5 |
| Ä 11 $a_2$ | | 20 | 50 | 25 | 5 | | | | 100 | 912,5 ± 20,0 |
| Ä 11 $a_8$ | | 17 | 42 | 27 | 9 | 4 | 1 | | 100 | 985,0 ± 27,5 |
| Ä $12_6$ | 1 | 36 | 49 | 10 | 3 | 1 | | | 100 | 827,5 ± 20,0 |
| Ä $12_7$ | 1 | 18 | 49 | 23 | 8 | | | | 100 | 922,5 ± 27,5 |
| Ä $15_2$ | | 34 | 50 | 16 | | | | | 100 | 835,0 ± 17,5 |
| | 3 | 217 | 323 | 124 | 26 | 5 | 1 | | 700 | (805) |

$P < 0{,}001$

It can thus be stated definitely that in *Triton*, too, the addition of one new genome involves a considerable increase of nuclear size. This increase is probably more than 50 % compared with the diploids.

Parallelly to this increased size of the cell-nuclei a reduction occurs in their absolute number per organ. In other words, the regulatory mechanism mentioned earlier is also found in *Triton*. The different organs are not larger in the triploids than in the diploids.

## MEIOSIS IN THE DIPLOIDS.

The normal meiosis in *Triton* has been described earlier by JANSSENS (1902), WALKER (1925) and others. Yet to a certain extent these publications must be considered superseded. Later investigations have been carried out by GALGANO (1933) and WICKBOM (unpublished). Meiosis, as a rule, is regular. The chromosome pairing and the development of chiasmata (=x-ta) during prophase I offer nothing remarkable. The number of x-ta per bivalent during diplotene is on the average 2,57 (WICKBOM). Metaphase I is regular with 12 bivalents and the number of x-ta per bivalent is on the average 2,02.

A detailed examination, however, reveals in each animal isolated minor abnormities, such as a few unpaired chromosomes in metaphase I, chromosome bridges, etc. Such minor deviations may be expected in all material of this kind, but it is not necessary to ascribe any special importance to them.

However, more definite changes in the course of meiosis may occur. Among the diploid *Triton* ♂♂ which were more closely examined one proved to have a meiosis of asynaptic type.

## DIPLOID ASYNAPTIC MEIOSIS.

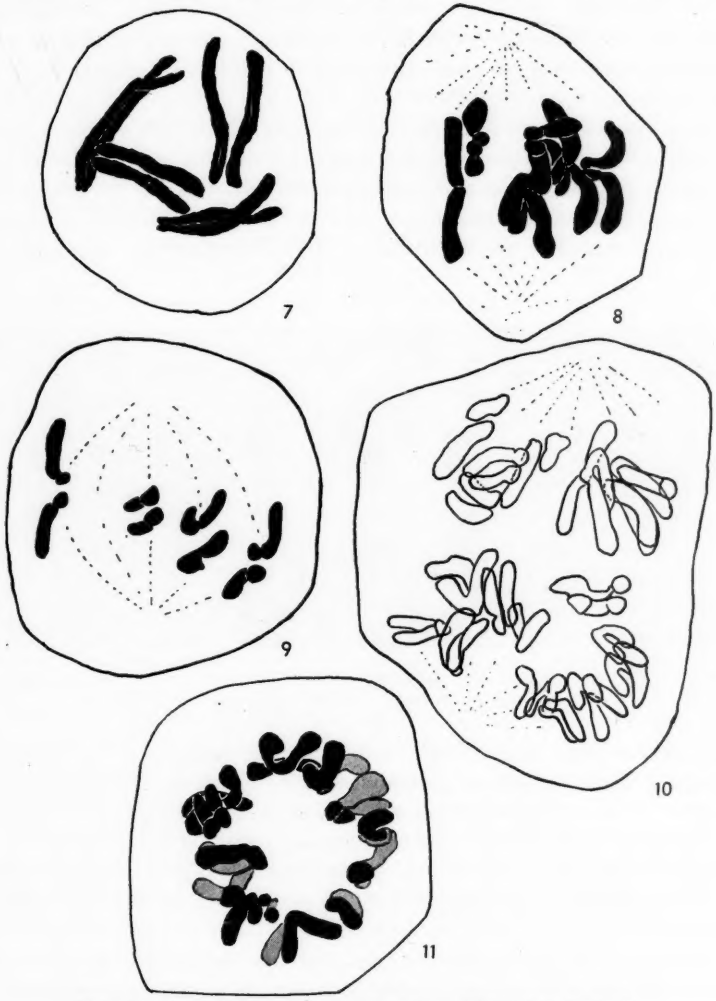
Asynapsis, i. e. a failure of chromosome pairing during the first meiotic division, has, as far as the author is aware, not been studied earlier in any vertebrate. On the other hand, the phenomenon is well known in some invertebrates, e. g., *Drosophila pseudo-obscura* and *Pygaera* hybrids (cf. DARLINGTON, 1937, p. 414) and above all in plant genetics. The best known examples are *Zea* (BEADLE, 1930, 1933), *Datura* (BERGNER, CARTLEDGE and BLAKESLEE, 1934), *Crepis* (RICHARDSON, 1935), *Pisum* (KOLLER, 1938), *Nicotiana* (GOODSPEED and AVERY, 1939), and *Allium* (LEVAN, 1940 a).

In the case under consideration here asynapsis was restricted to certain lobes of the testes. Some lobes had a completely normal appearance while others showed a more or less frequent occurrence of asynaptic divisions. Even in the most definite asynaptic lobes, however, a few completely normal first metaphases could be found. There was a sharp contrast between the normal and the asynaptic divisions. No transition forms were found as for the chromosome pairing. The number of x-ta during diplotene was thus either 0 per bivalent or the normal (about 2,6).

All the pachytene nuclei appeared quite normal. The pairing during pachytene was as complete as in the normal cells. In the transition to diplotene, however, the difference from the normal was striking. X-ta were wholly lacking (cf. Fig. 7). The chromosomes, however, lay in pairs, which is probably due to the earlier pairing and partly also to relational coiling. During the development to the metaphase stage the chromosomes fall further apart and appear as 24 univalents lying more or less at random on the spindle. At the same time there is a contraction which seemed to be just as strong as in normal meiosis. In any case, the chromosomes were decidedly shorter than in mitosis. In the fully developed metaphase the univalents are divided into their chromatids but are still connected at their undivided centromeres (cf. Fig. 8). The spindle always appeared quite normal. The centromere orientation was in some cases quite regular and all the chromosomes took up an equatorial position. In other cases, again, some of them were scattered over the spindle. In about a hundred examined cells only one bivalent was found. The asyndesis was thus practically complete.

It is difficult to interpret the continued course of division. Probably development takes place along several different lines. In many cases it was clearly seen that the centromeres divide at the beginning

of anaphase I. This is true of the univalents, which orientate themselves in the equatorial plane (cf. Fig. 9). Each half of the univalent




Figs. 7—11. Meiosis of asynaptic type. Discussion in text.

then moves to a different pole during the continued anaphase. Those univalents which do not orientate themselves in the equatorial plane pass undivided to the nearer pole. The distribution seems in some cases

to be rather equal in that about 24 chromatids (of which, however, some may connect 2 and 2 at their undivided centromeres) pass towards each pole (cf. Fig. 10). Two interphase nuclei of about the same size can thus be formed. In other cases when several of the univalents are scattered over the spindle a restitution nucleus is probably formed.

The second division is more difficult to follow. It probably only takes place in those cases where a restitution nucleus was formed at the first division. In such a case two diploid spermatids are formed at the second division if no centromere division has taken place during anaphase I. Otherwise the distribution is irregular during anaphase II.

12 13 14

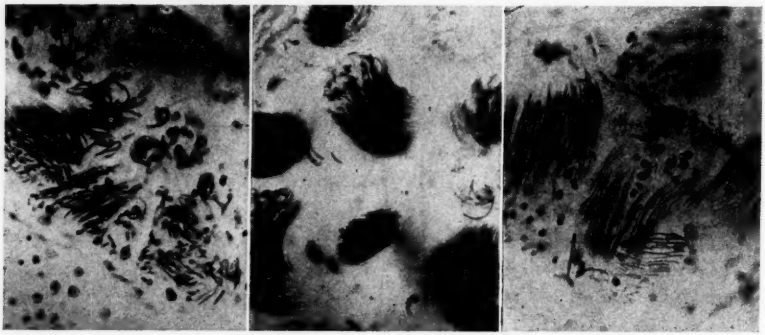


Fig. 12. Sperms in asynaptic lobulus. — Fig. 13. Do. in normal diploid. — Fig. 14. Do. in triploid. — Photomicrographs × 160.

Fig. 11 has been interpreted as a second metaphase with 24 single elements, but no anaphase corresponding to this configuration has been found. As most chromosomes lie in pairs in this metaphase, it may be assumed to have arisen after a non-homotypical first division. It is probable that the centromeres cannot divide more than once during meiosis and that a metaphase of this type passes over into a restitution nucleus.

Thus, asynapsis in *Triton* causes highly irregular divisions. The result ought to be spermatids which are either balanced diploids or have unbalanced chromosome numbers.

It is possible to observe during the continued spermatogenesis that some nuclei pass over into clear pyknosis, others develop further into highly abnormal spermatozoa (cf. Fig. 12).

For the present it is impossible to decide the cause of this asynapsis in *Triton*. It may quite well be controlled genotypically (somatic mutation?), but this is a pure assumption.

As a consequence of the asynaptic meiosis an extensive gamete selection can be observed (cf. Fig. 12). It is, however, not possible to prove quite satisfactorily to what this elimination of certain gametes is due. It is undoubtedly most probable that the cause lies in the chromosome sets being unbalanced either in number or otherwise. The causal connection behind the asynapsis itself may also influence the spermatogenesis.

Now, it is equally possible that asynaptic meiosis may also occur in *Triton* ♀♀. Here it may lead to the production of diploid egg-cells. As everything seems to point to these being fertilizable, a simple explanation may be found of the triploids which occur sporadically in nature. FANKHAUSER (1938 a) found his triploids partly among larvae which had performed their embryonic development, including fertilization, in water at room temperature and partly among those which had been exposed to an uncontrolled cold shock. It is extremely interesting that all the larvae (4 specimens) arose from the same ♀, which may indicate that there had been some irregularity in the meiosis.

The type of asynapsis here found in *Triton* is thus characterized by normal pachytene pairing without x-ta, complete asyndesis, and alternatively irregular or regular divisions according to the orientation at metaphase I. The combination of normal pachytene pairing and complete asyndesis agrees with LEVAN's *Allium* case (1940 a) and is a further proof that pairing during pachytene, however normal it may be, does not necessarily lead to the formation of x-ta (compare DARLINGTON's conception, 1937, p. 405). There do not seem to be other similarities to *Allium*, which after the absence of the first division forms regular dyads with somatic chromosome numbers.

The occurrence of asynapsis may be one explanatory basis for the formation of the abnormal spermatozoa which have been described in several vertebrates. As regards the origin of such spermatozoa, it is interesting that BROMAN (1911, p. 19) had already come upon the idea that the cause lay in an irregular distribution of the chromosomes during meiosis (*Homo*). He writes: »Wenn man aber die Entwicklung solcher Spermien (Riesen- und Zwergspermien) an Schnittpräparaten studiert, zeigt es sich, dass sie aus Präspermiden entstehen, deren Chromosomen nach den beiden Spindelpolen ungleich verteilt werden».

The occurrence of abnormal spermatozoa in man may in some

cases *a priori* be assumed to be due to asynaptic meiosis. It is of considerable speculative interest that a total asynapsis in the whole testis may explain certain forms of sterility (azoospermy). For several organisms it has been shown that asynapsis may be genotypically controlled (*Crepis*, *Zea*, etc.; cf. DARLINGTON, 1937, p. 405). LENZ (1936, p. 500) writes: »Fälle von Azoospermie, die nicht durch äussere Schädlichkeiten wie Gonorrhöe oder Röntgenstrahlen verursacht sind, sind als erbbedingt anzusehen». An interesting analogous conclusion is that certain types of sterility in man may quite simply be referred to a genotypically controlled asynaptic meiosis. Such a speculation ought to be interesting as a suggestion for further investigations in this sphere of human pathology.

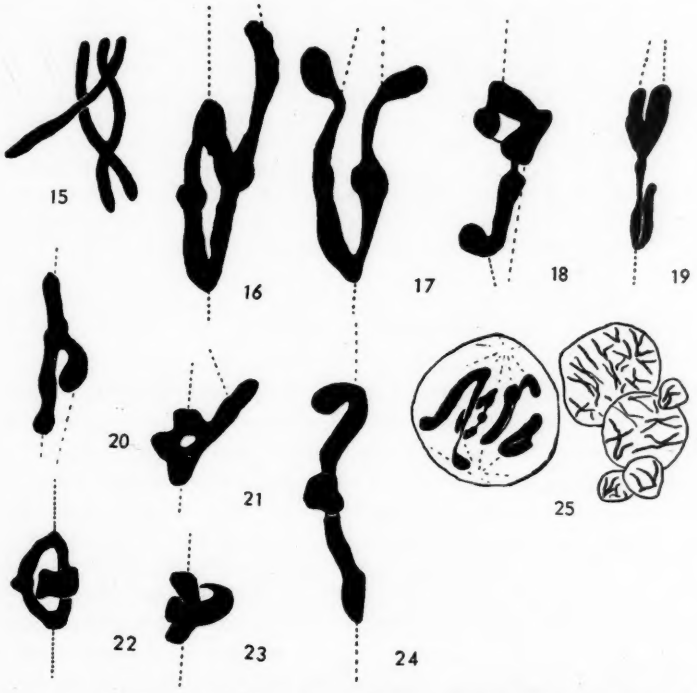
## MEIOSIS IN TRIPLOID TRITON.

The triploid meiosis offers much of interest, especially as it has not been previously studied in any vertebrate. *A priori* one must expect to find a course resembling that found in auto-triploid plants.

The early prophase stages do not seem to differ from conditions known in plants (cf. DARLINGTON, 1937, p. 85 *et seq.*). Thus, pairing takes place according to rule between two of the three homologous chromosomes. For this reason, associations appear during pachytene of either three partly paired or of two completely paired chromosomes. In the latter case the pairing between two homologues has been completed before the third has managed to join up and this has therefore remained unpaired. During diplotene normal chiasmata appear between the paired chromosome arms (cf. Fig. 15).

Metaphase I has been studied in detail. Typical trivalents, bivalents and a varying number of univalents appear. The trivalents are of the types shown in Fig. 26. Their x-ta are usually completely terminalized. Figs. 16—21 show the different types of trivalents which were found. In comparison Figs. 22—24 show some common bivalent types in a diploid. In 23 completely analysed cells 3—9 trivalents per cell were found as well as 3—8 univalents. The different configurations are shown in Table 8. It is remarkable that in about 50 % of the cells $\Sigma$ bivalents + trivalents exceed 12. This must indicate either that x-ta are formed between non-homologous chromosomes or that there is a partial homology between the chromosomes of the haploid set. The former assumption is less probable, being in complete opposition to the present conception of chromosome mechanics. According to this,

homology is a necessary condition for chromosome pairing and chiasma formation. A partial intra-haploid homology which can only assert




Figs. 15—21. Meiosis in triploid. 15: diplotene. 16—21: different types of trivalents. — Figs. 22—24. Different types of bivalents in normal diploid meiosis. — Fig. 25. Micro-nuclei after I.M. in triploid.

itself under exceptional conditions is the most probable explanation. There are, however, other possibilities. If the triploid has arisen from a diploid that was either a reduplication homozygote or an interchange heterozygote, there should be a possibility of intra-haploid pairing (DARLINGTON, 1937, p. 157). This autosyndesis in triploid *Triton* leads to the assumption that bivalents might appear in haploid animals. Unfortunately, it has not been possible up to now to keep any haploid alive so long that meiosis could be studied.

Fig. 26. Trivalent types.

During the first division the univalents are distributed at random in

TABLE 8. *Metaphase I.*

| Configuration | Frequency | Total number of bivalents + trivalents |
|---|---|---|
| $9_{III} + 3_{II} + 3_{I}$ | 4 | 12 |
| $7_{III} + 6_{II} + 3_{I}$ | 1 | 13 |
| $7_{III} + 5_{II} + 5_{I}$ | 1 | 12 |
| $7_{III} + 4_{II} + 7_{I}$ | 1 | 11 |
| $6_{III} + 7_{II} + 4_{I}$ | 2 | 13 |
| $6_{III} + 6_{II} + 6_{I}$ | 2 | 12 |
| $6_{III} + 5_{II} + 8_{I}$ | 1 | 11 |
| $5_{III} + 8_{II} + 5_{I}$ | 4 | 13 |
| $5_{III} + 7_{II} + 7_{I}$ | 2 | 12 |
| $4_{III} + 9_{II} + 6_{I}$ | 3 | 13 |
| $4_{III} + 8_{II} + 8_{I}$ | 1 | 12 |
| $3_{III} + 10_{II} + 7_{I}$ | 1 | 13 |

Total number of cells 23

| Number of univalents | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| Frequency | — | — | — | 5 | 2 | 5 | 5 | 4 | 2 | — |

M = 5,31

| Number of trivalents | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|
| Frequency | 1 | 4 | 6 | 5 | 3 | — | 4 |

M = 5,9

the cells. Those that lie near the equator orientate themselves here during the anaphase and divide, forming lagging chromosomes (predivision of the centromeres). Other univalents are distributed at random among the anaphase groups, i. e. they pass to the nearest pole undivided. In some rare cases the division of the univalent centromeres takes place already during metaphase I (cf. Fig. 27). Those which divided at the first division remain undivided during the second one.

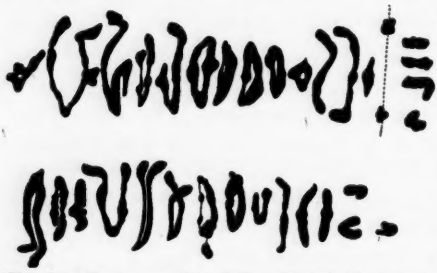


Fig. 27. Two complete I.M. of triploid. Upper row division of univalents at I.M.

The number of x-ta and their distribution between trivalents and bivalents are shown in Table 9. It should be observed that the chiasma frequency is lower in the triploids than in the diploids (the difference is statistically significant). Calculated in number of half-chiasmata per chromosome $2x = 2{,}02$ and $3x = 1{,}25$.

TABLE 9.

| Number of X-ta | Univ. | Bival. | Trival. | | Total X-ta |
|---|---|---|---|---|---|
| 0 | 122 | — | — | | |
| 1 | — | 64 | — | | 64 |
| 2 | — | 85 | 125 | | 420 |
| 3 | — | — | 11 | | 33 |
| Total | 122 | 149 | 136 | | 517 |
| Total chromosomes | 122 | 298 | 408 | = | 828 |

Number of $^1/_2$ X-ta per chromosome = 1,25

Each cell contains on the average 5,9 trivalents, 6,5 bivalents and 5,3 univalents.

*Metaphase II.*

| Number of chromosomes | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Frequency .. | 1 | — | — | 1 | 1 | — | 1 | 1 | 1 | 3 | 2 | 3 | 5 | 3 | 3 | 2 | — | 3 |

M = 15,0

In many cases the univalents are not affected by the anaphase movements. Instead, they surround themselves with their own nuclear membrane and form subhaploid micro-nuclei (cf. Fig. 25).

As more than 8 univalents have not been observed during metaphase I, it is probable that the chromosome numbers of these micro-nuclei do not as a rule exceed 8. Yet judging from the nuclear size most of them should not have more than a few chromosomes. Besides, there is little chance that all the univalents should be included in one nucleus. Most of the micro-nuclei seem to be approaching rapid degeneration. This is especially true of the very smallest. Yet in some cases the micro-nuclei could be seen preparing for a new division, which, however, probably cannot overcome the metaphase stage. No second anaphase with a subhaploid chromosome number has been found. The way in which the micro-nuclei are formed makes it highly probable that centrosomes are lacking in these cells. It may therefore be assumed that this is the reason why a normal second division cannot take place. Yet a quite normal metaphase orientation does take place. This may indicate that the centrosome, which is chiefly a kinetic anaphase organ, is not absolutely necessary for the orientation of the chromosomes to the metaphase plate. In spite of the absent anaphase separation, however, it is possible that the centromeres divide and a restitution nucleus with a doubled number of chromosomes is formed.

It seems clear that more than two divisable nuclei cannot be formed at the first division. Analogous conditions are found in some *Pygaera* hybrids with a complete lack of pairing (cf. DARLINGTON, 1937, p. 414).

Here, too, only two main nuclei, which must be assumed to contain centrosomes, can carry out a second division. The micro-nuclei are eliminated. In plants, on the other hand, it seems as if such micro-nuclei formed of univalents are able to carry out a second division as well. Thus ÖSTERGREN (1940), examining the meiosis of the hybrid *Agropyron junceum* × *A. repens*, found such micro-nuclei in anaphase configuration. If these investigations are representative of animals and plants respectively, they argue against the occurrence of centrosomes in plants.

In metaphase II a varying number of chromosomes are found (cf. Table 9). Chromosome numbers between 12 and 21 can easily be explained as a consequence of the orientation in metaphase I, and the only metaphase II with four chromosomes shown in Table 9 is probably a micro-nucleus. The difficulty then is to explain metaphases II with 7, 8, 10 and 11 chromosomes. These may originate from micro-nuclei incapable of overcoming the stage of metaphase II. At this point those centromeres which have not pre-divided, post-divide, and the chromosome number may increase.

As a consequence of the chromosome distribution during the triploid meiosis spermatids are formed with varying chromosome numbers and accordingly also with a varying nuclear size. A comparison between the size of the diploid and triploid *spermiocyte nuclei* shows the following. Both types have practically a constant size. The averages for $r$ (= radius) and $V$ (= volume) are given in Table 10. As is seen, the newly added third haploid set has involved an increase of the nuclear volume by about 50 %.

In the diploids the *spermatid nuclei* are all about the same size, with extremely small variations. On the other hand, in the triploid there is a very great variability. In the table there are 510 spermatid nuclei from the triploid. Their size has primarily been calculated without regard to the structure of the nuclei. It is interesting that among the nuclei with a diameter less than 7 units are those with a clearly degenerated structure. The chromatin is condensed and there is a sign of incipient or complete pyknosis. The number of pyknotic nuclei increases considerably with diminished size. Correlating chromosome number and nuclear size in this case, one would like to assume that the nuclei with a diameter of less than 7 units are subhaploid, as the normal haploid spermatids have a diameter that does not fall below these 7 units. This would then favour the idea that most of the subhaploid spermatids will not survive. The question is, however, complicated by the fact that

TABLE 10. *Nuclear size of spermatids in diploid and triploid.*

| | Diam. in units (unit = 1,1 $\mu$) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | n | M | $r$ | $r^3$ |
| Triploid. Total ..... | 13 | 52 | 22 | 9 | 16 | 11 | 12 | 63 | 187 | 88 | 30 | 6 | 1 | | 510 | 8,21 | | |
| Triploid. Pycnotic nuclei | 11 | 47 | 19 | 5 | 6 | 2 | | | | | | | | | | | | |
| Triploid. Normal nuclei | 2 | 5 | 3 | 4 | 10 | 9 | 12 | 63 | 187 | 88 | 30 | 6 | 1 | | 420 | 9,33 | 4,67 | $101,5 = r_1^3$ |
| Diploid ..... | | | | | | | 12 | 188 | | | | | | | 200 | 8,44 | 4,22 | $75,2 = r_2^3$ |

$r_2^3 : r_1^3 = 1 : 1,35$

*Nuclear volume of spermiocytes in diploid and triploid.*

| | $r$ | $V$ | $V_{2n} : V_{3n}$ |
|---|---|---|---|
| 2n | 8,25 $\mu$ | 2350,89 $\mu^3$ | 1 : 1,5 |
| 3n | 9,35 $\mu$ | 3422,19 $\mu^3$ | |

the pyknosis as such involves a shrinking of the nucleus. The correlation between its size and chromosome number, therefore, cannot be estimated with certainty. However, the accumulation of pyknoses in the region of the very smallest nuclear classes makes it probable that at this stage it is mostly the subhaploid nuclei which degenerate.

In an autotriploid organism with 36 chromosomes the chromosome numbers in metaphase II might be expected to vary between 12 and 24. The average number ought to be 18. Now here an average of 15 is found. This value, however, is uncertain because of the small number of cells analysed. Still, it may be explained on the basis of the tendency of the univalents to form micro-nuclei.

As the spermiocyte nuclei with 36 chromosomes are about 50 % larger than those with 24 (cf. Table 10), one might expect nuclei with an average of 18 chromosomes to be 50 % larger than those with 12. Here, however, the average of the triploid is 15, and if, knowing the uncertainty involved, we correlate the chromosome number and the nuclear size, the increase in size should be about 25 %. Here, when measured, an increase is found of about 35 %. Thus, if from this latter result the conclusion is drawn that the spermatids of the triploid have on the average fewer than 18 chromosomes, it seems to be correct in this case. Since the measurements are based on a relatively large number of cells, and the two calculations give the same result, it is justifiable to assume that there is a tendency to form spermatids with a chromosome number that is near the haploid.

An argument of this kind must always be more or less schematic. It is not possible to fit in all the deviations which are theoretically possible. Consequently, it is not possible to present any exact proof. It should therefore be pointed out that to refer the spermatid chromosome number to the configurations of metaphases II is not strictly adequate. It has been done owing to the difficulty of finding a sufficient number of good anaphase configurations. The metaphases which have 12 or more chromosomes pass over into regular anaphases. The spermatids that are then formed must have the same chromosome number as the metaphase plate showed. Yet the relatively few subhaploid metaphases II may cause deviation. Thus, a restitution nucleus may be formed after centromere division. The consequence would be spermatids with an increased number of chromosomes (see above). If this is the case, the average number of chromosomes per spermatid will be increased to over 15. Assuming that all the subhaploid cells in Table 10 form restitution nuclei with a doubled chromosome number,

the average number of chromosomes per spermatid should be 16,3. In accordance with the explanation given above this should mean an increase of size by 36 % on the average. Thus the uncertainty caused by the subhaploid nuclei does not seem to affect the essential conclusion, viz. that there is a clear tendency to form spermatids with a chromosome number near that of the haploid.

The examination of the spermatogenesis proper, i. e. the development after the second meiotic division, shows clearly that a very large number of gametes are not able to survive (cf. Fig. 14). This may be due partly to the chromosome number being too high or too low, partly to the chromosome sets being unbalanced and, finally, partly to lethal combinations of genes. Degenerations owing to influences from the surrounding tissue are less probable.

Different types of cellular degeneration are found. In the most extreme cases an early pyknosis occurs, possibly combined with a karyorhexis. In other cases the development of the spermatozoa is not broken off until a later stage. Then the spermatid nuclei have, like the normal ones, passed over from the spherical to an elliptical shape and attempts at differentiation into head and tail are seen. Then, however, the development is stopped and the chromatin is condensed so that the nuclei become quite compact.

If those testicular lobes are studied in which the spermatogenesis has reached the final stage plenty of degenerated forms are found (cf. Fig. 14). The picture shows a striking contrast compared with conditions in a normal diploid testis (cf. Fig. 13). In spite of the fact that only one with 12 chromosomes was found among the 30 analysed metaphases II, *morphologically normal spermatozoa were observed in quite a large number*. Whether these are also normal physiologically, it is not possible to state. As, however, spermatogenesis as such is a very sensitive physiological process, one is justified in assuming that some of these spermatozoa would have been able to function. Highly unbalanced chromosome *numbers* probably lead to gamete selection (haplontic sterility), which is not in conflict with an assumption that animal gametes in general can bear greatly unbalanced *gene* complexes (cf. DOBZHANSKY, 1937, p. 268).

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Institute of Genetics, Lund University, January, 1944.

## SUMMARY.

(1) As an introduction a short review is given of polyploidy investigation in animals, with special reference to the vertebrates.

(2) Of 129 egg-cells which were exposed to low temperature in close connection with insemination, 48 developed to larvae. Among these 5 were triploid and 1 haploid. Further, 3 embryos were haploid. No case of haploidy or triploidy was found among the controls.

(3) Of 64 egg-cells exposed to low temperature more than half an hour after insemination but before the first cleavage, 13 gastrulated and developed normally to the neurula stage. At the cytological examination it could be ascertained that these embryos were built up like mosaics of cells with different chromosome numbers (multiform aneuploidy).

(4) Embryos exposed to low temperature at the neurula stage showed, as a consequence, a metaphase block in their cell divisions. Centromere division occurs at metaphase and restitution nuclei may be formed with an increased number of chromosomes.

(5) The effect of the low temperature on the unsegmented egg-cell is discussed. Besides the temperature and the duration of the cold shock, the internal constitution of the egg-cell when the experiment is started must be of decisive importance for the effect. The induction of haploidy, triploidy and multiform aneuploidy ought to be referable to a destructive effect on the spindle. An essential condition seems to be that the low temperature treatment should be able to suppress the activity of the spindle during the time between two centrosome and centromere divisions.

(6) Haploidy, induced by cold shock, involved a development of dwarf larvae with greatly reduced viability and a definite tendency to oedema.

(7) The triploid larvae showed no certain increase of body size compared with the diploids. Nor was there any difference in development or general viability.

(8) Measurements of a large number of cell-nuclei in diploid and triploid animals showed great individual variations. In triploid animals the nuclei of the erythrocytes, cartilage and nerve cells were on the average 68 %, 74 % and 63 %, respectively, larger than in the diploids in the material at disposal. As regards the spermiocyte nuclei in a spontaneously developed triploid, they were found to be about 50 % larger than the corresponding nuclei of some diploids.

(9) In connection with the increase in nuclear and cell volume the number of cells per organ decreases. This means that the different organs retain about their normal size.

(10) Meiosis in diploid ♂♂ is, as a rule, regular, although one case with considerable changes in its course was found. The abnormity has the character of an asynaptic meiosis, a type of meiosis which has not been described earlier in any vertebrate. In this case it was characterized by normal pachytene pairing without chiasmata, complete asyndesis, and either regular or irregular divisions according to the orientation in metaphase I. The result is an extensive elimination of nuclei and the development of abnormal spermatozoa.

(11) Meiosis in a triploid *Triton* ♂ is described. The course corresponds fairly well to that found in an autotriploid organism. In metaphase I there are on the average 5,9 trivalents, 6,5 bivalents and 5,3 univalents. A formation of micro-nuclei during the first division is relatively usual. In metaphase II chromosome numbers varying from 4 to 21 are found. There seems to be a definite tendency to form spermatids with a chromosome number near that of the haploid. A very large number of spermatid nuclei undergo pyknosis, others develop into abnormal spermatozoa.

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# MEIOSIS IN ANEMONE APENNINA WITH SPECIAL REFERENCE TO CHIASMA LOCALISATION

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## INTRODUCTION.

ACCORDING to DARLINGTON (1937 a, p. 110), *Anemone apennina* and a few other plant species are characterized by a high degree of chiasma localisation. In the case of *Anemone apennina* this classification is based upon the illustrations in a preliminary paper on the cytology of the genus *Anemone*, which was made by the present writer in his student-years (BÖCHER, 1932). Undoubtedly the treating of the very interesting *Anemone apennina* was not exhaustive and deserves completion, so much the more as some errors of interpretation are to be found in the preliminary paper.

*Material and methods.* — The material of *Anemone apennina* is from the Bontanical Gardens in Copenhagen. Flower buds were fixed in NAVASHIN's fluid after a short immersion in CARNOY. Staining was done by NEWTON's iodine-gentian violet technique. Additional studies were made by using smears.

## SOMATIC CHROMOSOMES.

In *Anemone apennina* 16 chromosomes are counted in somatic divisions. The somatic complement consists of four pairs with median (or slightly submedian) attachment constrictions, two with subterminal and two with almost terminal constrictions. In some of the chromosomes (e. g. the centre one in the cell Fig. 1 *c*) spiral structure was visible, and in some cases one of the arms contained a small sector with a lower staining capacity. One of the medially and two of the subterminally constricted pairs often show no chromatid splitting at a moment when the other chromosomes are distinctly split up and mainly held together by the centromeres (Fig. 1 *c*, 2 *o*).

Resting nuclei were studied in order to count the heterochromatic parts of the chromosome complement. The number of large stainable

bodies (chromocentres) varied from 12 to 18, 15 and 16 being the most frequent numbers (Fig. 1 *a*). The counting was made difficult by the occurrence of a number of small stainable granules. In tapetal tissues a number of cells with 32 chromosomes occurred, and corresponding resting stages contained about 30 large chromocentres (Fig. 1 *b*). One or two of the very small chromocentres were clearly attached to the nucleolus and connected with a larger chromocentre by thin threads

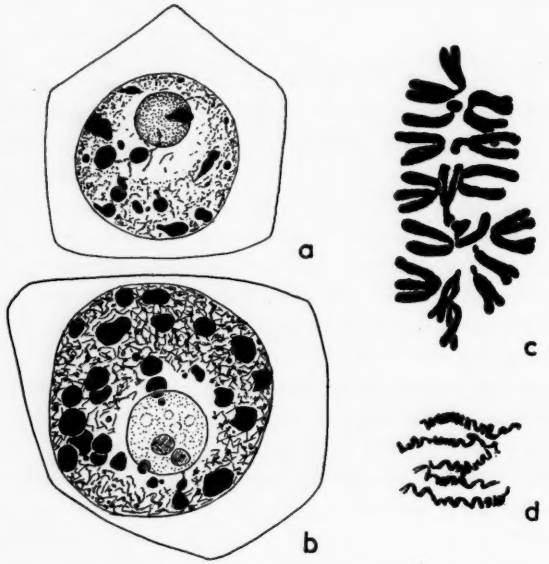


Fig. 1. *a*: A normal diploid resting cell from the pollen sac tapetum with about 15 large chromocentres and a number of small stainable bodies. — *b*: A tetraploid cell from the tapetum with about 30 large chromocentres and a number of small stainable bodies. — *c*: Somatic metaphase with unusually condensed chromosomes; 4 pairs with median constrictions, 2 with subterminal, and 2 with almost terminal constrictions. — *d*: Part of the chromosomes of a late-telophase nucleus touched by the knife and showing spiral structure. — × 2100.

(Fig. 1 *b*). In haploid resting nuclei in the pollen grains there are 7—9 large chromocentres.

Spiral structure was observed in resting cells or, more correctly, in very late telophases. Where the cells were cut over, the knife had uncoiled the spirals in such a way that the joint spirals of two chromatids were visible (Fig. 1 *d*). In the tapetal cells of a *Paeonia* species I have found a similar spiral structure in the same stage.

## MEIOSIS.

The material included all stages from leptotene to second telophase (Fig. 2 *a*—*r*). In early pachytene 14—16 deeply stained parts of the chromosomes were counted (Fig. 2 *c*). These parts are made up of condensed and large chromomeres. Sometimes (Fig. 3 *a* in the upper left-hand corner) they closely resemble the proximal condensed parts in *Fritillaria* (DARLINGTON, 1935), but in most cases they are thicker and more homogeneous, owing perhaps to a greater content of matrix. Sometimes it is possible to detect two such dark bodies in the same pair (Fig. 3 *a*). They are sometimes separated by rather large uncondensed gaps. In other cases they lie rather close together and may perhaps correspond to the condensed parts on either side of the centromeres in *Fritillaria*. Unfortunately, it is not possible to study the pachytene bivalents in their entire lengths. What can be observed is that the condensed parts are sometimes placed near the ends of the chromosomes and sometimes at a rather long distance from the ends. During the early stages of diplotene the condensation usually includes about 28 free ends of the 8 bivalents (Figs. 2 *e*, 3 *b*). In the medial-submedially constricted pairs the ends are clearly in advance of the median parts. In these chromosomes the pachytene condensation seems to have commenced at a point lying some distance from the end and to have later continued towards the ends. Undoubtedly, the primarily condensed parts in *Anemone apennina* are not always proximal as in the case of *Fritillaria* and others. Among the 16 condensed parts of the pachytene chromosomes 8 (six single and two made up of two parts close to one another; cf. Fig. 3 *b*) presumably correspond to the 16 heterochromatic bodies found in the resting nuclei. The other precocious parts must be euchromatic.

In some of the slides the pachytene chromosomes had another appearance, the double threads being thicker and more deeply stained without the differential condensation described above. The threads appeared to be enveloped in a matrix. In the same slides the chromosomes at metaphase I were unusually pale and transparent.

The pachytene pairing does not always seem to be complete. Thus, it can be seen that some of the synapsed pairs have segments where the threads are only lying parallel, and sometimes the distal ends are unpaired (Fig. 3 *a*). One of the pairs is attached to the nucleolus with two deeply stained very small bodies, which are most often clearly separated. In a few pachytene nuclei two nucleoli were observed, and in resting cells and at leptotene two nucleoli are not rare. The nucleolus

left at diplotene disappears, or it may survive till diakinesis. In the late anaphases of the first and second division no nucleoli are visible, but in stages which may be denoted as early resting stages several nucleoli seem to be formed (Fig. 2 *r*).

In mid-diplotene the median parts of the long chromosomes have

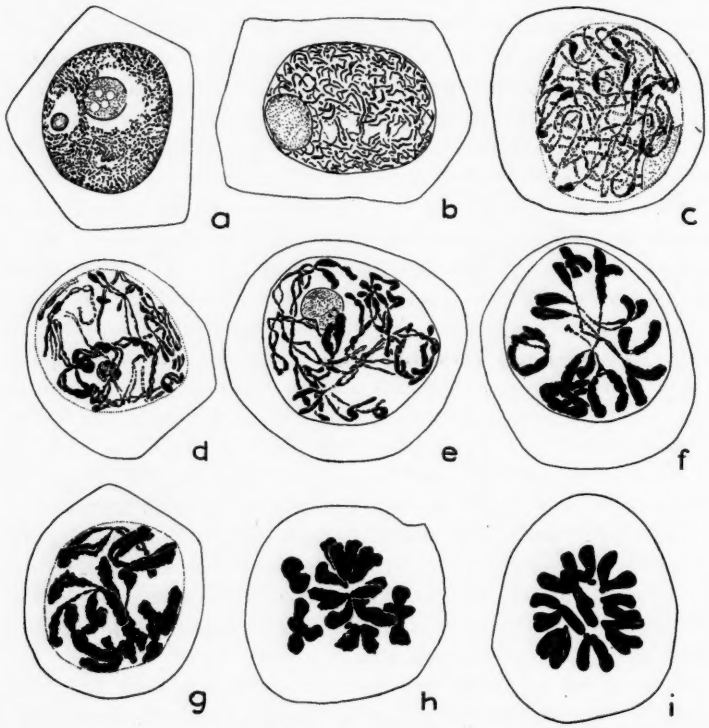


Fig. 2. The normal course of meiosis. *a*: resting stage, *b*: leptotene, *c*: pachytene (15—16 deeply stained parts visible), *d*: early diplotene, *e*: mid-diplotene, *f*: late diplotene, *d—f*: showing the progressive condensation, *g*: diakinesis, *h*: prometaphase, *i*: metaphase, polar view.

a structure almost like that of the threads at pachytene, while, as already mentioned, the distal parts are condensed. The diplotene separation is very pronounced near the precocious condensed ends. At late diplotene the thickening increases considerably, and in the medial-submedially constricted pairs the condensation now develops in the direction of the centromere. The chiasmata are clearly localised to the

uncondensed segments. Even cells at diakinesis frequently contain bivalents with uncondensed parts.

The number of chiasmata decreases from about 3—5 at mid-diplotene to 1—2 at metaphase. Owing to difficulties in distinguishing relational coiling from crossing-over, an exact counting of the chiasmata

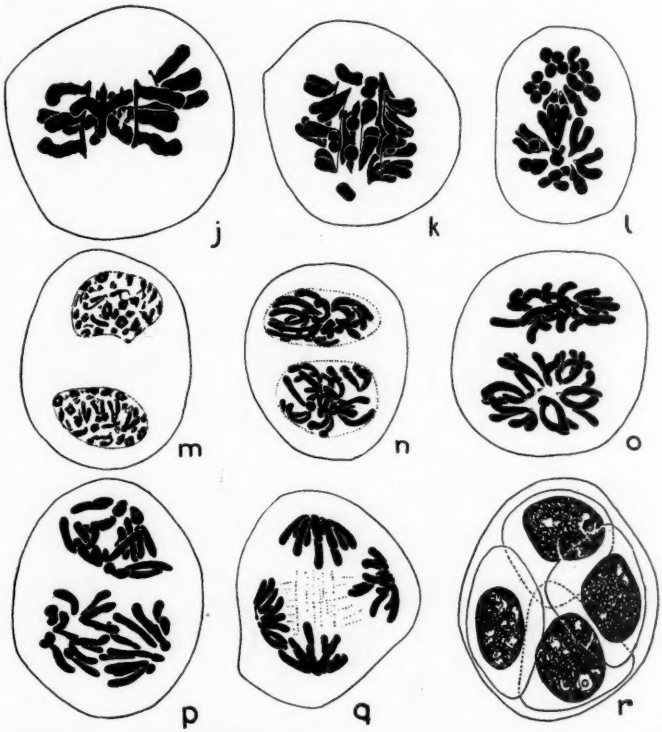


Fig. 2 (continued). — *j*: metaphase, side view, *k*: early anaphase, *l*: late anaphase, *m*: interphase, *n*: second prophase (or early prometaphase), *o*: second metaphase, *p*: second anaphase, *q*: second telophase, *r*: young pollen tetrad. — × 1700.

could not be made. The chiasma frequency may often be relatively low in the long pairs, but it seems to vary rather considerably within the same pair from cell to cell. At diakinesis the reduction in the number of chiasmata has ceased. The chiasmata left are always interstitial in the long pairs and sometimes terminalised in the short pairs. The reduction in the number of chiasmata here observed is parallel to that described in other species of *Anemone* (MOFFETT, 1932), *Pulsatilla*

(ROSENTHAL, 1936), and *Ranunculus* (LARTER, 1932). These authors suppose the chiasma localisation to be brought about through a delayed onset of terminal affinity, the chiasmata slipping off the ends of the chromosomes. If, however, in the long pairs of *Anemone apennina* two

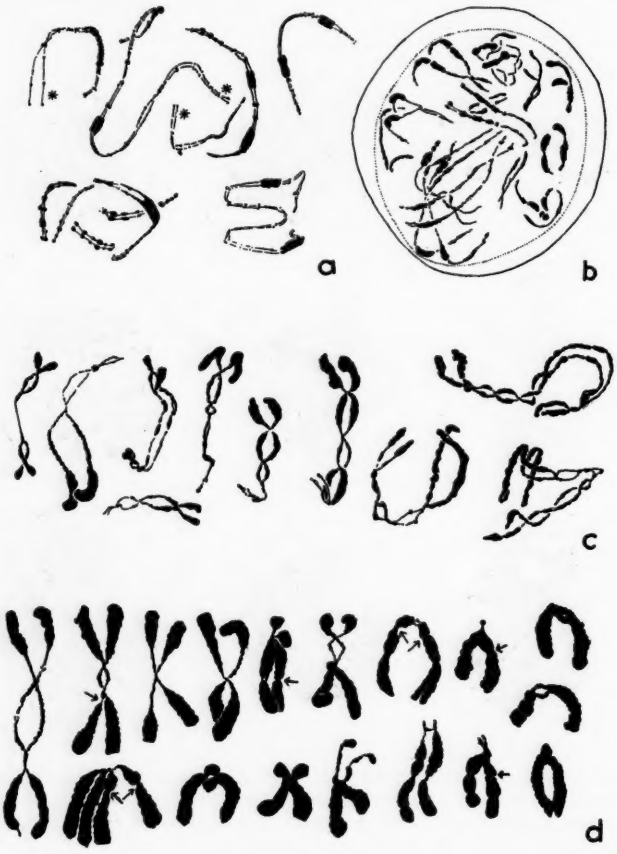


Fig. 3. *a*: Parts of the pachytene threads showing chromomeres and two kinds of deeply stained parts. Ends of the bivalents are marked with *. — *b*: Mid-diplotene, strongly differentiated material, only the precocious condensed parts stained. — *c*: Bivalents at mid-diplotene; some of the short ones are possibly only ends of the long bivalents which have four precociously condensed ends. — *d*: Bivalents at diakinesis or late diplotene (the long pair on the left with two chiasmata). The third and fourth bivalent from the left in the upper row were placed close to one another in the same cell. The probable positions of the primary constrictions are marked with arrows. — × 2100.

chiasmata are present at mid-diplotene, they seem to move towards the central parts of the bivalents (Fig. 3 *d*, to the left). Such a view was suggested by DARLINGTON (1929), but was later abandoned by him. In *Anemone apennina* this hypothesis is supported by the fact that condensation in the long pairs at this stage develops from the ends towards the central parts. Very occasionally distal, terminalised chiasmata occur in the long bivalents at metaphase (Fig. 4 *r*—*v*). They are probably formed in uncondensed ends distal to the condensed parts. These parts may in the case of *Anemone apennina* determine the direction of

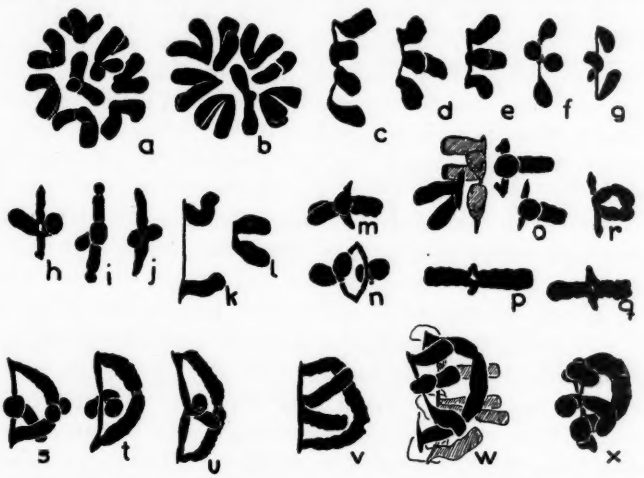


Fig. 4. *a*—*b*: polar views of metaphase I, *c*—*l*, *o*—*q*: normal bivalents with one chiasma, *c*—*e*: typical E-bivalents, *o*: three short bivalents with interstitial chiasmata surrounding one E-bivalent. *m*—*n*, *r*—*u*: bivalents with two chiasmata, the types *r*—*u* very rare. *v*: unequal bivalent. *w*: one trivalent or ring of three chromosomes having probably arisen after translocation or interchange; the configuration surrounded by three large E-bivalents; the portion between the two left-hand centromeres extended and perhaps broken. *x*: quadrivalent or ring of four. — × 2100.

the chiasma movements. In *Allium fistulosum*, which has pronounced chiasma localisation (LEVAN, 1933, p. 230), a similar behaviour is described. LEVAN mentions the possibility that the different parts of the bivalents behave differently. Distal chiasmata may here be terminalised and slip off the ends while proximal ones are stationary or move inwards. Studies on later stages in *Anemone apennina* have revealed the occurrence of inversions; hence, a failure of terminalisation may perhaps in some cases be caused by lack of homology (cf. *Paeonia*; DARK, 1936).

As is the case in other plants with localised chiasmata (e. g. *Fritillaria meleagris*, NEWTON and DARLINGTON, 1930; *Allium fistulosum*, LEVAN, 1933), late diplotene, diakinesis and metaphase are characterized by the large number of free distal elements. Figs. 2 *h—j* and 4—5 illustrate the appearance of the bivalents at metaphase. As compared with *Fritillaria meleagris* and *Allium fistulosum*, the number of bivalents with one interstitial or terminal chiasma is very high. In the cell Fig. 5 showing a side view of the bivalents all pairs have only one chiasma. In the species of *Fritillaria* the twelve bivalents have 2—4, rarely 1 chiasma, and in *Allium fistulosum* 2—3, rarely 1 or 4 chiasmata are left at metaphase. The almost terminally or subterminally constricted pairs form bivalents which have the shape of a body with two wings (Fig. 4 *o—q*). The medial-submedially constricted pairs form bivalents of the shape of an E with two median lines. In these E-bivalents the portions between the chiasma and the centromeres are extended and narrow.

Bivalents with two chiasmata are never frequent. Of these the cruciform type, which dominates in *Allium fistulosum*, may also be the most frequent type in *Anemone apennina* (Fig. 4 *m—n*). The type Fig. 4 *r* is very exceptional and has only been observed twice. Bivalents with more than two chiasmata were not seen and are presumably completely lacking. In one case a configuration made up of four chromosomes has been observed (Fig. 4 *x*), and in a few cases associations of three chromosomes occur (Fig. 4 *w*). Whether these configurations are to be interpreted as results of interchange or whether they represent true multivalents, could not definitely be made out.

The fact that the somatic chromosome complement of *Anemone apennina* consists of two sets each with four medial-submedially, two subterminally, and two almost terminally constricted chromosomes raises the question whether *Anemone apennina* is a polyploid species originating after doubling in a species or a species hybrid with the chromosome number $2n = 8$. Some other facts support this hypothesis. First, the chromosome numbers in the genera *Ranunculus* and *Adonis*, both belonging to the *Anemonoideae*, indicate that here the basic number is four (LANGLET, 1932). In the case of the tetraploid *Allium porrum* (LEVAN, 1940) the large E-bivalents are the results of a formation of quadrivalents which do not survive until metaphase. In *Anemone apennina* a parallel arrangement of two paired threads with deeply stained bodies of the same shape and size was observed in several cells at pachytene. Evidence of the formation of pachytene quadrivalents

was not obtained, nor were metaphase quadrivalents of the types described by LEVAN found, possibly because the number of chiasmata in *Anemone apennina* is usually one, whereas two chiasmata are present in *Allium porrum*. At late diplotene the large E-bivalents are composed of four almost equal arms, which in very many cases touch each other only at one point. In a number of cells two such bivalents lie very close together (Figs. 2 *f*, 3 *d*), resembling the diakinesis pictures in *Allium porrum* (LEVAN, l. c., Fig. 1 *e—f*), where the quadrivalents are held together only by torsions.

During the anaphase separation the bivalents behave very differently. Pairs with terminal chiasmata are precocious, while the large E-bivalents are somewhat lagging. In the cell Fig. 5 the separation is almost completed for four of the pairs with interstitial chiasmata, but in one large E-bivalent (that on the right) the separation has not yet commenced. The delay may in some cases be due to an inversion and, if so, the E-bivalents represent bridges from which the fragments have not yet separated (cf. UPCOTT, 1937, Figs. 10 *d* and 11). If the normal separation is completely arrested, a break of the narrow portions between the centromeres and the chiasma will result. This will lead to the formation of one large acentric configuration consisting of four chromatids. Two observations support this assumption. At early anaphase (Fig. 2 *k*) the threads between the chiasma and the centromeres are very narrow and sometimes almost strained to invisibility, as though the chiasma was prevented from moving. Furthermore, laggards made up of four chromatids (Fig. 6 *i*) occur. These seem to be entirely acentric, without the centromeres which in *Anemone apennina* are generally very distinct (Fig. 2 *j*, *l*, *o*). In *Allium Cepa* × *fistulosum* LEVAN (1936, pp. 201—202) describes strained bivalents with interstitial chiasmata which, as a result of structural changes, do not move, and MÜNTZING (1934) has observed a fragmentation caused by breakage of bridges in a *Crepis* hybrid (see e. g. MÜNTZING, l. c., Fig. 24 and his discussion on p. 295).

Fig. 5. Metaphase and early anaphase I. All bivalents separately drawn. In the lower cell one lagging E-bivalent. — × 2100.

## CONDENSATION, PAIRING DISTURBANCES AND STRUCTURAL CHANGES.

The most striking irregularities observed at the first metaphase and anaphase in *Anemone apennina* are described below.

*Condensation.* — In one pollen sac the chromosomes at the first metaphase were abnormally long and narrow (Fig. 7 *c*), resembling those of a second metaphase (Fig. 2 *o*), but otherwise of normal appearance with one or sometimes two chiasmata per bivalent. Another pollen sac in the same anther deviated considerably, showing results of pairing disturbances of different kinds (see later) combined with reduced shortening and thickening of the chromosomes. DARLINGTON (1937 b) mentions a graded developmental variation in the condensation of the chromosomes in the pollen grains of *Fritillaria* and *Tradescantia.* Pollen sacs with intermediate condensation in *Anemone apennina* indicate that a similar, graded variation occurs; here, however, it is meiosis which is affected.

*Number of chiasmata.* — The normal appearance of the long medially constricted pairs is the E-bivalent. In by far the most pollen sacs this bivalent type is completely dominant (see e. g. Fig. 2 *j*), but in one sac the E-bivalents in a number of cases were converted into the type illustrated in Fig. 4 *s*—*u*. All the bivalents Fig. 4 *s*—*v* come from this sac and are characterized by one interstitial and one terminal (very rarely subterminal) chiasma. The very occasional occurrence of configurations made up of more than two chromosomes is also limited to this sac.

*Inversions.* — The number of cells with bridges and fragments in first anaphase varies from very few in most pollen sacs to rather large numbers in a few sacs. In the most extreme case 12 per cent of the cells contained bridges and fragments, 18 per cent contained lagging chromosomes or fragments from which the bridges had disappeared, and 60 per cent looked normal. In the cell Fig. 6 *l* a fragment, two normal chromatids and a loop chromatid occur, the latter presumably giving rise to a second divisional bridge (cf. RICHARDSON, 1936, Fig. 16 and FRANKEL, 1937, Figs. 9—10). Typical bridges with fragments (long or short) are shown in Fig. 6 *a*—*e*. The large fragments are found persisting at second telophase or late anaphase (Fig. 6 *k*). The other cells in Fig. 6 deviate greatly and are not easy to explain. Thus, in Fig. 6 *f* one bridge is seen but no typical fragment. On the other hand a large and possibly acentric configuration lies close to the bridge.

Apparently it is made up of four connected parts, but it may most probably be interpreted as a large acentric ring with two rather long free ends. At one point it carries two small bodies. In the cell Fig. 6 *j* one small body of the same kind is attached to one of the two frag-

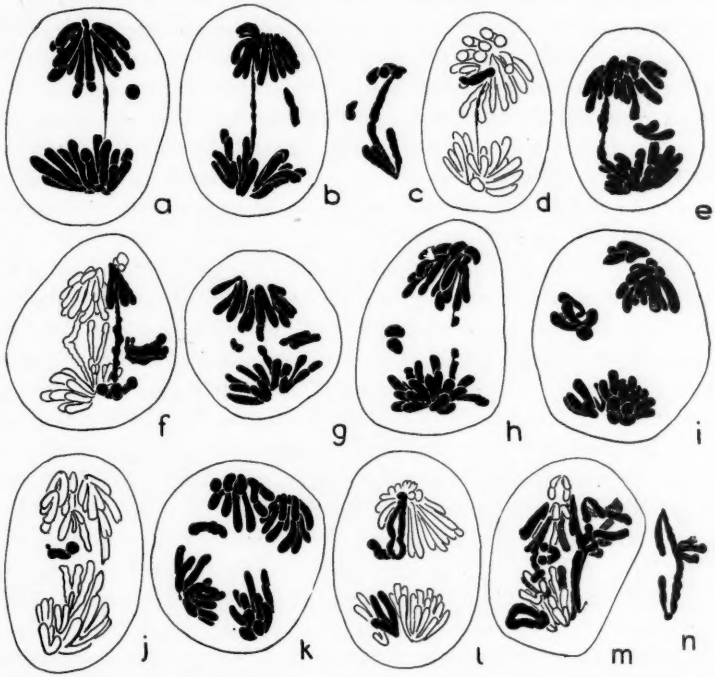


Fig. 6. Inversion and fragmentation. — *a—e*: one acentric fragment and one bridge, *f*: one bridge and a large possibly acentric configuration (cf. text), *g*: one fragment and a short broken bridge, one dividing univalent, *h*: one of the chromosomes has a short distal segment separated from the rest; it may be a broken bridge; two rather large fragments of the same size, *i*: one V-shaped fragment from which the bridge has disappeared and one acinetic configuration perhaps made up of four chromatids and having arisen after breakage of a lagging E-bivalent, *j*: two fragments and perhaps two broken bridges, *k*: second telophase, one fragment, *l*: two normal and a loop chromatid and one fragment, *m*: two normal and a loop chromatid and a short fragment; one acinetic V-shaped fragment and a dicentric bridge, two bridge-like laggards, the lower one drawn separately in Fig. 6 *n*; see furthermore text. — × 1700.

ments. These small trabant-like bodies are somewhat reminiscent of the so-called T-phenomena in rye described by KATTERMANN (1939) and PRAKKEN and MÜNTZING (1942). Perhaps some parts of the chromosomes which have formed the acentrics are attracted by the pole with-

out being able to move the lagging configurations. In the greatly deviating cell Fig. 6 *m* one bridge with a rather long U-shaped fragment, one loop chromatid with a short fragment and to the right a complex of two laggards may occur. It is unfortunately impossible to clear up the structure of this complicated configuration. It may be a separating multivalent association. It looks as if the two bridge-like laggards have three median arms, and they are possibly connected by a fourth arm. Furthermore, it is not impossible that connections between some of the median arms and a fifth chromosome exist or have existed. In the slide one of the laggards is covered by the other; it is drawn separately in Fig. 6 *n*. At a point (marked with +) a third arm is elongated and seems to be connected with one of the arms on the fifth chromosome in the upper corner to the right.

*Other structural changes, asyndesis.* — The cell Fig. 6 *n* must be a result of rather complicated structural changes. In the above-mentioned anther with reduced condensation two of the pollen sacs contained cells showing large pairing disturbances and structural changes of different kinds. Fig. 7 *d*—*e* are two examples of the appearance of entire cells, and Fig. 7 *f*—*k* show some of the configurations found. Undoubtedly, normal bivalents are very rare, and structural changes combined with asyndesis make the pictures very complicated. It is possible that the bivalent Fig. 7 *g* contains an inversion, and the same is the case with the unequal bivalent in Fig. 4 *v*. which, however, is from a more normal pollen sac. In Fig. 7 *j* a chiasma is formed probably between homologous segments in different arms of the chromosomes (cf. Fig. 28 in RICHARDSON, 1936); the two other chiasmata are interstitial or terminalised as in the bivalents Fig. 4 *s*—*v*. In Fig. 7 *k* one or perhaps two arms are connected with a translocated part of another chromosome. Some of the long V- or U-shaped chromosomes in the Fig. 7 *d*—*e* are probably unpaired chromosomes with median constrictions. In the pollen sacs with normal chromosome condensation partial asyndesis and fragmentation were observed in a single cell (Fig. 7 *a*). Another cell in the same sac contained a fragment (Fig. 7 *b*). In very many normal sacs at first metaphase only a few such cells with one fragment were observed. The fragments were of the same size as the short acentrics which had arisen after crossing-over in inversions (see Fig. 6 *a*).

*Cytomixis.* — In the greatly deviating anther described above many of the cells were exceptional, due to cytomixis. In a number of cases two cells were connected by chromosome elements almost as described

by MÜNTZING and PRAKKEN (1941). Cytomixis, which in the genus *Anemone* has been described from an anther with abnormal position in *A. ranunculoides* and from the hybrid *A. nemorosa* × *ranunculoides* (BÖCHER, 1932), was in the case of *A. apennina* mainly found in some of the many anthers containing cells at leptotene and pachytene. A similar localisation of cytomixis to single anthers or loculi occurs in several species of *Ranunculus* (e. g. *R. Traunfellneri*) and in rye (MÜNTZING and PRAKKEN). Undoubtedly, the irregular metaphases in the deviating anther are to some extent results of cytomixis in early

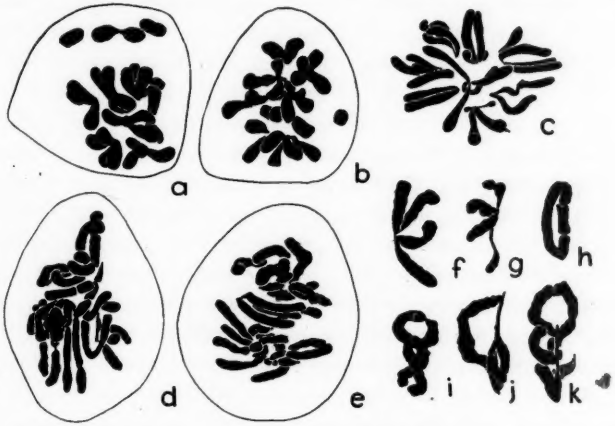


Fig. 7. *a—b*: metaphase I, two cells with normal condensation showing results of asyndesis and fragmentation, *c*: metaphase I in a pollen sac with reduced condensation, *d—e*: two cells resulting from abnormal pairing and structural changes combined with reduced condensation, *f—k*: some of the configurations from the greatly deviating cells in the pollen sac with abnormal pairing, etc.; cf. text. — × 1700.

stages, but it is not probable that the structural changes and the asyndesis are connected with cytomixis, which here may mainly be an accompanying phenomenon.

## DISCUSSION.

Several attempts to explain the localisation of chiasmata have been made. British cytologists (DARLINGTON, DARK, LARTER, and MOFFETT) have proposed a number of hypotheses suggesting that the localisation is mainly due to chromosome morphological factors, *viz.* (1) that the pairing in the distal parts of very long chromosomes is incomplete owing to the »time limit for pairing», (2) that the terminalisation is

arrested by change of homology, and (3) that the terminalised chiasmata slipp off the ends of the chromosomes owing to lack of »terminal affinity». Furthermore, the possibility that some of the chiasmata move towards the centromere has been mentioned by LEVAN (see above).

Of special interest to the problem are the cytological observations of the hybrid *Allium Cepa* (with random chiasmata) × *A. fistulosum* (with localised chiasmata). According to LEVAN (1941), random chiasmata predominate in both $F_1$ and $F_2$ of this cross. This indicates that the chiasma behaviour is largely determined by the genotype.

In *Anemone apennina* a case of pronounced chiasma localisation has occurred in a species with structural abnormalities, and, therefore, a discussion on the significance of a lack of homology to the localisation is of some interest. First, however, it will be necessary to mention the main agencies for the production of structural changes.

Experimental studies have revealed that chromosome interchange, inversions, fusions, fragmentations as well as asyndesis may be induced by temperature changes (SAX, 1937). [A summary of the literature on this subject (OEHLKERS, STRAUB and others) is contained in the review by KÜSTER (1942, pp. 28—30).] The effect of temperature treatment recalls that of X-ray treatment. In *Anemone apennina* a similar effect of temperature is rather probable, seeing that the material was collected outside the natural area of the species. Owing to the special structure of the flower the same stage occurs in different stamens at different points of time, and, therefore, under varied environmental conditions. The rather different chromosome behaviour in different anthers may be caused by temperature changes or perhaps by a different supply of various substances (food, growth hormones, etc.) within the spiral of sexual organs. In *Ranunculus reptans* a corresponding difference between anthers belonging to the same bud was observed in material from Greenland (at the northern limit of the species). Here, however, the spindle was also influenced (BÖCHER, 1938).

If environmental or physiological factors are the main agencies in the production of structural changes, the localisation of chiasmata is hardly due to lack of homology. On the other hand this possibility cannot be excluded. If inversions were constantly present, an increase in the chiasma frequency caused by environmental factors would probably result in the persistence of one or more chiasmata in the inverted segments, at points where normally none are left. The environment may affect either the pairing, the chiasma movements, and the terminal forces in such a way that constantly present structural abnormities

are disclosed, or it may directly create the structural changes at leptotene.

According to LEVAN (1933) and FRANKEL (1937), no inversions or other structural irregularities have been observed in their material of the two species *Allium fistulosum* and *Fritillaria meleagris*, both of which have localised chiasmata. It would undoubtedly be an attractive task to expose these species to extreme temperatures. If no structural irregularities were found, this would indicate that a lack of homology was of no importance to the question of chiasma localisation.

LEVAN (1941) discusses the importance of chiasma localisation to the cytological stability of the tetraploid derivatives of the hybrid *Allium Cepa* × *fistulosum*: »By selection of types with many localised chiasmata it should be possible to arrive at a tetraploid *Cepa* × *fistulosum* with strict chiasma localisation», and »by utilisation of such a property as chiasma localisation it may thus be possible to obtain a decidedly greater stability than is ever found in a polyploid form with ordinary random chiasmata». The idea is that types with localised chiasmata are more fertile than types with random chiasmata and, consequently, a selection of the former may even occur in nature. In the case of *Anemone apennina* the evolution of the chromosomes with localised chiasmata may have commenced by structural changes induced by environmental factors having become constant and resulted in the selection of biotypes with strict chiasma localisation.

## SUMMARY.

The early ancestors of *Anemone apennina* may be supposed to have had a haploid set of 4 chromosomes. On this view the species should be classified as dibasic (cf. GEITLER, 1934, p. 277). But owing to hybrid origin or structural changes the two original sets of four chromosomes are not identical and the plant behaves as a monobasic species.

A special case of differential condensation is described. In the long chromosomes the distal parts are in advance of the median parts. At mid- or late diplotene the chiasmata are localised to the uncondensed regions. As a rule, only one chiasma per bivalent is left at metaphase. This is interstitial in the long pairs and sometimes terminalised in the short pairs. The direction of the chiasma movements is discussed.

The long bivalent with one interstitial chiasma resembles an E with two median lines. At anaphase some of the E-bivalents are lagging. This behaviour may be due to lack of homology. The possibility of

the formation of large acentric configurations resulting from breaks in the extended parts of these E-bivalents is mentioned.

Owing to exceptional environmental or physiological influences the chromosome behaviour is abnormal in a few anthers. The irregularities concern the condensation, the number of chiasmata, the frequency of inversions and other structural changes as well as the pairing. In one anther more than 12 per cent of the cells contained inversion bridges or, rarely, loop chromatids.

The significance of lack of homology to the problem of chiasma localisation is discussed.

Copenhagen, April, 1944.

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# ABSTRACTS — KURZE MITTEILUNGEN

OLOF TEDIN: Small samples of a Poisson series.

In his »Statistical methods for research workers» FISHER presents a method by which it is possible to ascertain whether the variation within small samples agrees with the theoretical distribution of a Poisson series. For each sample an index of dispersion is calculated by the formula $\frac{S(x-\bar{x})^2}{\bar{x}}$ and, if the samples belong to a true Poisson series, the indices are distributed as $\chi^2$ for $n$-1 degrees of freedem, if $n =$ the number of variates in the sample. For a single sample the test is of small value, but if a large number of samples have been obtained under comparable conditions the distribution of the indices may be used to test the reliability of the methods used. If the nature of the material studied is such that the variation should follow a Poisson series but the distribution of the indices does not agree with $\chi^2$, it may be concluded that the method of sampling or of analysis is at fault.

FISHER (l. c., sect. 16) recommends the method for, e. g., counts of bacterial colonies in dilution plates where »we may have only 5 parallel plates, bearing perhaps 200 colonies apiece». The present author has tried to apply the method to material where by routine a large number of different but comparable biological populations are sampled, the $n$ of the samples varying from 2 upwards but usually being less than 10. In this material the $m$ of the presumed Poisson series is usually low, rarely above 2 and often less than 1. On such material the method of FISHER cannot be used indiscriminately.

The relative frequencies of the variate values 0, 1, 2, etc. for a few low values of $m$ are given below.

Relative frequencies of variate values

| $m$ | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| 0,1 | 0,9048 | 0,0905 | 0,0045 | 0,0002 | | | | | | |
| 0,5 | 0,6065 | 0,3033 | 0,0758 | 0,0126 | 0,0016 | 0,0002 | | | | |
| 1,0 | 0,3679 | 0,3679 | 0,1839 | 0,0613 | 0,0153 | 0,0031 | 0,0005 | 0,0001 | | |
| 2,0 | 0,1353 | 0,2707 | 0,2707 | 0,1805 | 0,0902 | 0,0361 | 0,0120 | 0,0034 | 0,0009 | 0,0002 |

When $m$ is as low as 0,1 and $n = 2$, no less than $(0{,}9048)^2$ or 81,9 % of all samples will show the values 0,0. Even if $n = 10$, $(0{,}9048)^{10}$ or 37 % of the samples will show only the value 0. For this low value of $m$ there is no agreement with the distribution of $\chi^2$ unless, perhaps, when $n$ is large enough to allow a direct comparison with a Poisson series. For any value of $m$ and any value of $n$ the relative frequencies of samples with the index of dispersion = 0 may be obtained by summing the $n$th powers of the relative frequencies of the variates 0, 1, 2, etc. For three values of $m$ tabulated above these frequencies are as follows:

Relative frequencies of dispersion indices = 0 when $n$ is equal to

| $m$ | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| 0,5 | 0,4658 | 0,2514 | 0,1438 | 0,0847 | 0,0506 | 0,0304 | 0,0184 | 0,0111 | 0,0067 |
| 1,0 | 0,3084 | 0,1060 | 0,0377 | 0,0136 | 0,0048 | 0,0018 | 0,0006 | 0,0002 | |
| 2,0 | 0,2068 | 0,0486 | 0,0123 | 0,0031 | 0,0008 | 0,0002 | | | |

This simple test shows that the frequency of low values of the index is altogether too large, when $m$ is small, if $n$ is not sufficiently large, but it also indicates that with increasing values of $m$ the frequencies of indices $= 0$ drop off rapidly. In a few cases the total distribution of the index has been calculated, with the result that when the value 0 is in excess the rest of the distribution also differs widely from that of $\chi^2$.

It would be of considerable value to know at which combinations of $m$ and $n$ the distribution of the indices reaches so fair an agreement with that of $\chi^2$ that the test of FISHER may be used with reasonable safety. The author has made one attempt in this direction by calculating the distribution when $m = 2$ and $n = 4$. The 4th power of the decanomial showing variate frequencies of at least 0,0001 when $m = 2$ includes 715 terms. Of these 244 have frequency values of at least 0,0001, the sum of these frequencies is 0,9915 and the remaining 0,0085 is obviously the sum of frequencies below 0,00005. The index value has been determined for each of the 244 terms mentioned, and these indices have been grouped in accordance with the $\chi^2$ table of FISHER. Multiplying the frequencies of each group so as to make their sum $= 1$, the values given below have been obtained. The corresponding frequencies of the $\chi^2$ distribution are given for comparison.

Frequencies of dispersion indices between the values

| | 0,352 | 0,584 | 1,005 | 1,424 | 2,366 | 3,665 | 4,642 | 6,251 | 7,815 |
|---|---|---|---|---|---|---|---|---|---|
| index | 0,0377 | 0,0367 | 0,1405 | 0,0476 | 0,2423 | 0,1820 | 0,1181 | 0,1202 | 0,0384 | 0,0361 |
| $\chi^2$ | 0,05 | 0,05 | 0,10 | 0,10 | 0,20 | 0,20 | 0,10 | 0,10 | 0,05 | 0,05 |

The agreement is fair but not good. The fraction 0,0123 of the indices is 0, whereas only 0,01 of $\chi^2$ is less than 0,115. In spite of this slight excess in the very lowest class there are too few index values below the limit 0,352 that indicate the 0,05 point of the $\chi^2$ distribution. The distribution of the indices is markedly discontinuous, and if the classes are narrow this becomes very apparent. Small changes in the value of $m$, however, may change the frequencies in neighbouring classes considerably. This indicates the possibility that, whereas the distribution of indices disagrees with that of $\chi^2$ when $m$ and $n$ are small, the sum of indices, obtained from a large number of samples from biological populations with varying true values of $m$, may agree with the $\chi^2$ value appropriate to the accumulated number of degrees of freedom. As a matter of fact, in a material of 44 samples with altogether 191 degrees of freedom the sum of the indices was 192,50, in close agreement with the theoretical value of $\chi^2$. In 10 samples with $n = 2$, the sum of indices was 4,48, in 8 samples with $n = 3$, the sum of indices was 16,91, etc., the sum of indices in all cases falling within reasonable limits.

The difficulty offered by samples all the variates of which are $= 0$ should be pointed out. It is impossible to know whether such samples represent a population with the true value of $m = 0$, or whether they are random samples of populations with higher values of $m$. If they are all included in a comprehensive test, they may wrongly increase the frequency of low values of the index; if they are excluded, the result will most probably be a deficiency in such low values.

Not being a mathematician, the author has made no further attempts to solve the problem here barely outlined. It would certainly be of great value if a modification adequate to small values of $m$ and $n$ could be given of the test of FISHER. The Poisson series seems to be comparatively little used in genetical research, but if its true possibilities became more generally known, there is no doubt that it might be used in many instances where less adequate or no statistical methods at all are now employed.

LARS EHRENBERG: The shape of the spindle at metaphase is conditioned by the shape of its molecules.

In a series of experiments not yet completed the shape of the spindle at metaphase has been studied on the basis of statistical methods. It was found that the curvature of the outline of the spindle as it appears when the spindle is studied at right angles to the direction of the nuclear division was a measurable property. The material consisted of fixed and stained root-meristems of *Salix fragilis* × *alba*.

The investigations have so far demonstrated: (1) The curvature of the outline of the spindle increases with a fall of temperature within the range 30° to 10° C. (2) The curvature of the outline of the spindle is greater in the cells of the plerome than in those of the periblem. Simultaneously it was observed that the hydrogen ion activity in the plerome cells was about 0,1 of that of the periblem cells. (3) Treatment of the roots with small concentrations of substances which in higher concentrations have a colchicine effect induces a greater curvature of the outline of the spindle. So far it has been demonstrated that colchicine and ethyl alcohol have this property. Pure hydrocarbons likewise show a tendency in this direction.

It can be demonstrated that the greater curvature of the outline of the spindle is most likely due to a contraction and thickening of its constituents, which consist of oblong polypeptide chains. In connection with this observation the chemical powers which might induce the contraction of the protein molecules may be discussed. The results of the investigation suggest that the formation and breaking up of hydrogen bonds whithin and between the polypeptide chains have an important function in this process. These bonds most likely arise between the $>$ CO and $>$ NH groups of the main polypeptide chains as well as between the oxygen and nitrogen carrying side-chains. Other powers, however, may also play a part in the contraction of the molecules. Among these the attraction between the lipophil groups within the molecules, a power which according to ÖSTERGREN's hypothesis might explain the colchicine-mitosis, seems to play the most important part.

Although conclusive evidence has so far not been furnished, several factors suggest that the speed of the anaphase movements of the chromosomes is lowest in the spindle that shows the greater degree of curvature in its outline. At the present stage of the investigations it may, therefore, be assumed that the breaking up and reformation of hydrogen bonds play some part in the process of mitosis, a hypothesis which needs further verification.

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